

**EXHIBIT A**  
**PENDING CLAIMS CORRELATED**  
**WITH CLAIM NUMBERS IN SERIAL NO. 08/872,222**

**Former Claim 11:**

11. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 12:**

12. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(mono-phosphate) analogue.

**Former Claim 13:**

13. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(di-phosphate) analogue.

**Former Claim 14:**

14. The phosphoinositide analogue of claim 13, wherein said phosphoinositide analogue is a PtdIns(4,5)P<sub>2</sub> analogue.

**Former Claim 15:**

15. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(poly-phosphate) analogue.

**Former Claim 16:**

16. The phosphoinositide analogue of claim 11, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement.

**Former Claim 17:**

17. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by derivatization.

**Former Claim 18:**

18. The phosphoinositide analogue of claim 17, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is alkyl, substituted alkyl or alkenyl.

**Former Claim 19:**

19. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form 2-OAc.

**Former Claim 20:**

20. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is CH<sub>3</sub>.

**Former Claim 21:**

21. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is ω-amino-alkyl.

**Former Claim 22:**

22. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N-substituted-ω-amino-alkyl.

**Former Claim 23:**

23. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N,N-disubstituted-ω-amino-alkyl.

**Former Claim 24:**

24. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by replacement.

**Former Claim 25:**

25. The phosphoinositide analogue of claim 24, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyhalo or 2-dideoxyhalo phosphoinositide analogue.

**Former Claim 26:**

26. The phosphoinositide analogue of claim 25, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyfluoro phosphoinositide analogue.

**Former Claim 27:**

27. The phosphoinositide analogue of claim 11, wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue.

**Former Claim 28:**

28. The phosphoinositide analogue of claim 27, wherein a reporter group is incorporated.

**Former Claim 29:**

29. The phosphoinositide analogue of claim 28, wherein the reporter group is a photoaffinity reporter group.

**Former Claim 30:**

30. The phosphoinositide analogue of claim 28, wherein the reporter group is a fluorescent reporter group.

**Former Claim 31:**

31. The phosphoinositide analogue of claim 28, wherein the reporter group is a spin probe reporter group.

**Former Claim 32:**

32. The phosphoinositide analogue of claim 28, wherein the reporter group is a radioactive label reporter group.

**Former Claim 33:**

33. The phosphoinositide analogue of claim 28, wherein the reporter group is a stable isotope label reporter group.

**Former Claim 34:**

34. The phosphoinositide analogue of claim 27, wherein a conjugand is incorporated.

**Former Claim 35:**

35. The phosphoinositide analogue of claim 34, wherein the conjugand is alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl,  $\omega$ -thio-(alkyl-C=O) or  $\omega$ -thio-alkyl.

**Former Claim 36:**

36. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a polymer.

**Former Claim 37:**

37. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a chromatographic matrix.

**Former Claim 38:**

38. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a gold surface.

**Former Claim 39:**

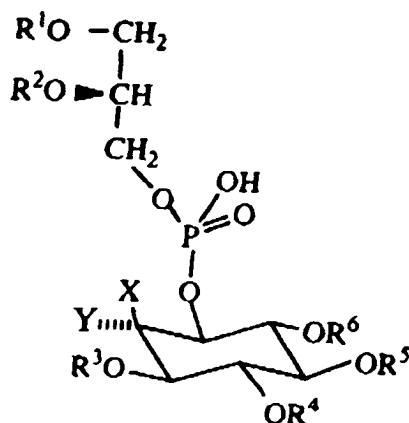
39. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a reporter group.

**Former Claim 40:**

40. The phosphoinositide analogue of claim 11, wherein one or both glycerol esters are replaced by ether bonds.

**Former Claim 66:**

41. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycerol-ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein:

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ ; or
- (c)  $X = Y = F$  or  $(=O)$ ;  
where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R, R' = \text{alkyl}$  or  $\text{alkenyl}$ ;

and wherein:

- (e)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (f)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (g)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (h)  $R^6 = H, P(=O)(O\text{-protecting group})_2, \omega\text{-aminoalkyl}, \omega\text{-aminoalkenyl}, \omega\text{-sulfhydrylalkyl}, \omega\text{-carboxyalkyl}, \omega\text{-(4-azidosalicylamido)-alkyl}, \text{alkyl-aminofluorophor}, \text{alkyl-amidofluorophor}, \text{or alkyl-fluorophor}.$

**Former Claim 70:**

42. The phosphoinositide analogue of claim 11, wherein:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; and
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 79:**

43. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 84:**

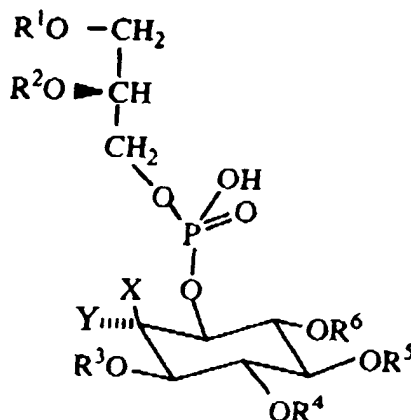
44. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate.

**Former Claim 85:**

45. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate.

**Former Claim 86, Amended as shown:**

46. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glyceric ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R = \text{alkyl, alkenyl, alkynyl}$ ,  $R' = \omega\text{-aminoalkyl, } \omega\text{-(substitutedamino)-alkyl, } \omega\text{-aminoalkenyl, } \omega\text{-sulfhydrylalkyl, } \omega\text{-carboxyalkyl, } \omega\text{-(4-azidosalicylamido)-alkyl, } \omega\text{-(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl}$ ; or where  $R' = \text{alkyl, alkenyl, alkynyl}$ ,  $R = \omega\text{-aminoalkyl, } \omega\text{-(substitutedamino)-alkyl, } \omega\text{-aminoalkenyl, } \omega\text{-sulfhydrylalkyl, } \omega\text{-carboxyalkyl, } \omega\text{-(4-azidosalicylamido)-alkyl, } \omega\text{-(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl}$ ; or where  $R = R'$ , except when  $R = R' = \text{alkyl}$ ;

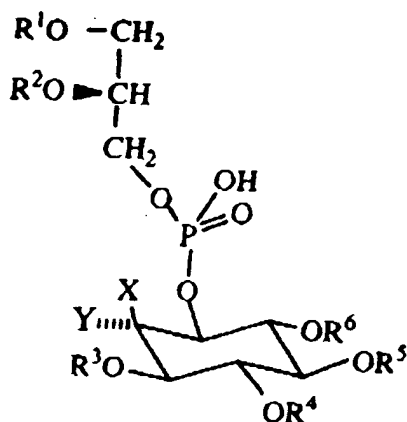
and wherein

- (c)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,  
 (d)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,  
 (e)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,

- (f)  $R^6 = H$ ,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 87:**

47. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glyceric ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
  - (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , or
  - (c)  $X = Y = F$  or  $(=O)$ ;
- where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, *N*-substituted- $\omega$ -aminoalkyl or *N,N*-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R = \text{alkyl}$ , alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' = \text{alkyl}$ , alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,



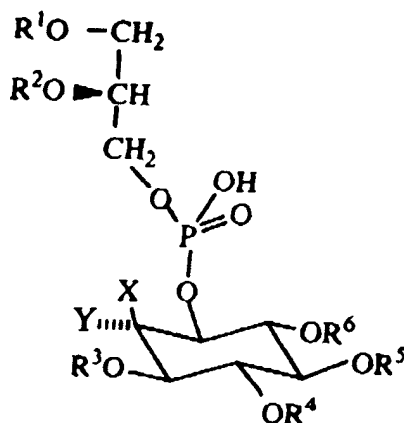
$\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (f)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (g)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (h)  $R^6 = H$ ,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 88:**

48. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
  - (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ ; or
  - (c)  $X = Y = F$  or  $(=O)$ ;
- where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

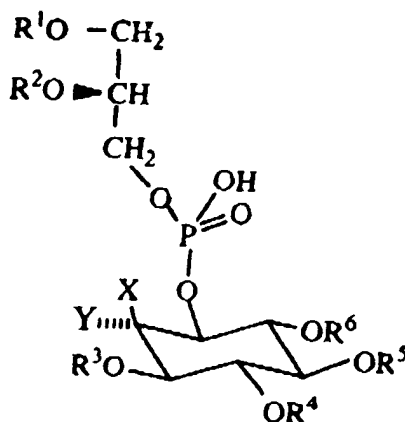
- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R, R' = \text{alkyl or alkenyl}$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$   
(f)  $R^4 = H$ , or  $P(=O)(OH)_2$   
(g)  $R^5 = H$ , or  $P(=O)(OH)_2$   
(h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 89, Amended as shown:**

49. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3, R^4, R^5, R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R = \text{alkyl, alkenyl, alkynyl}$ ,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' = \text{alkyl, alkenyl}$ ,

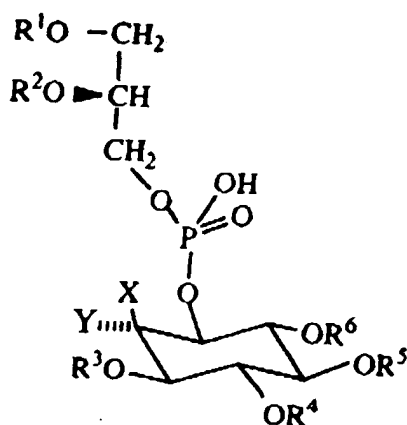
alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R = R', except when R = R' = alkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 90:**

50. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ ; or

- (c)  $X = Y = F$  or  $(=O)$ ;  
 where  $R =$  alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R =$  alkyl, alkenyl, alkynyl,  $R' =$   $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R =$   $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

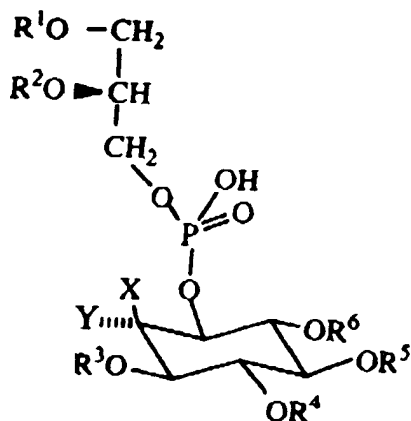
- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$   
 (f)  $R^4 = H$ , or  $P(=O)(OH)_2$   
 (g)  $R^5 = H$ , or  $P(=O)(OH)_2$   
 (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 91, Amended as shown:**

51. Matched pairs of the 2-modified phosphatidylinositol-phosphates of claim 48 and the corresponding phosphatidylinositol-phosphate structure lacking the 2-modification, wherein  $X=OH$  and  $Y=H$ , or  $X=H$  and  $Y=OH$ .

**New Claim, based upon former Claim 81, Amended:**

52. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, [alkyl-fluorophor], hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, hydroxylalkyl, or ketoalkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$   
(d)  $R^4 = H$ , or  $P(=O)(OH)_2$   
(e)  $R^5 = H$ , or  $P(=O)(OH)_2$   
(f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**New Claim, supported by claim 11:**

53. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or  
(b) a conjugand suitable for linking to a reporter group, polymer, chromatographic matrix, or gold surface is incorporated in the fattyacyl or inositol residue; wherein said conjugand is selected from the group consisting of  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl, hydroxylalkyl and ketoalkyl, and wherein the amino, substitutedamino, sulfhydryl, carboxyl, hydroxyl and keto functions are free and unsubstituted, or are covalently linked to a reporter group;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**EXHIBIT B**  
**PENDING CLAIMS**  
**CONTINUATION OF SERIAL NO.08/872,222 (NUBI:004--1)**

11. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

12. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(mono-phosphate) analogue.

13. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(di-phosphate) analogue.

14. The phosphoinositide analogue of claim 13, wherein said phosphoinositide analogue is a PtdIns(4,5)P<sub>2</sub> analogue.

15. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(poly-phosphate) analogue.

16. The phosphoinositide analogue of claim 11, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement.

17. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by derivatization.

18. The phosphoinositide analogue of claim 17, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is alkyl, substituted alkyl or alkenyl.

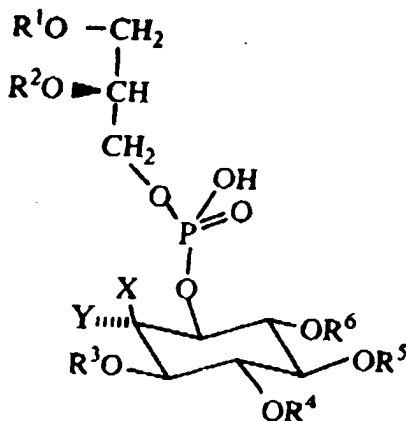
19. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form 2-OAc.
20. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is CH<sub>3</sub>.
21. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is ω-amino-alkyl.
22. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N-substituted-ω-amino-alkyl.
23. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N,N-disubstituted-ω-amino-alkyl.
24. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by replacement.
25. The phosphoinositide analogue of claim 24, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyhalo or 2-dideoxyhalo phosphoinositide analogue.
26. The phosphoinositide analogue of claim 25, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyfluoro phosphoinositide analogue.
27. The phosphoinositide analogue of claim 11, wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue.
28. The phosphoinositide analogue of claim 27, wherein a reporter group is incorporated.

29. The phosphoinositide analogue of claim 28, wherein the reporter group is a photoaffinity reporter group.
30. The phosphoinositide analogue of claim 28, wherein the reporter group is a fluorescent reporter group.
31. The phosphoinositide analogue of claim 28, wherein the reporter group is a spin probe reporter group.
32. The phosphoinositide analogue of claim 28, wherein the reporter group is a radioactive label reporter group.
33. The phosphoinositide analogue of claim 28, wherein the reporter group is a stable isotope label reporter group.
34. The phosphoinositide analogue of claim 27, wherein a conjugand is incorporated.
35. The phosphoinositide analogue of claim 34, wherein the conjugand is alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl,  $\omega$ -thio-(alkyl-C=O) or  $\omega$ -thio-alkyl.
36. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a polymer.
37. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a chromatographic matrix.
38. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a gold surface.
39. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a reporter group.



40. The phosphoinositide analogue of claim 11, wherein one or both glycerol esters are replaced by ether bonds.

41. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycerol-ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein:

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , and  $Y = H$ ; or  
 $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ ; or
- (c)  $X = Y = F$  or  $(=O)$ ;  
where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl,  
N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R, R' = \text{alkyl or alkenyl}$ ;

and wherein:

- (e)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (f)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (g)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,

- (h)  $R^6 = H, P(=O)(O\text{-protecting group})_2, \omega\text{-aminoalkyl}, \omega\text{-aminoalkenyl}, \omega\text{-sulfhydrylalkyl}, \omega\text{-carboxyalkyl}, \omega\text{-(4-azidosalicylamido)-alkyl}, \text{alkyl-aminofluorophor}, \text{alkyl-amidofluorophor}, \text{or alkyl-fluorophor}.$

42. The phosphoinositide analogue of claim 11, wherein:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; and  
(b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

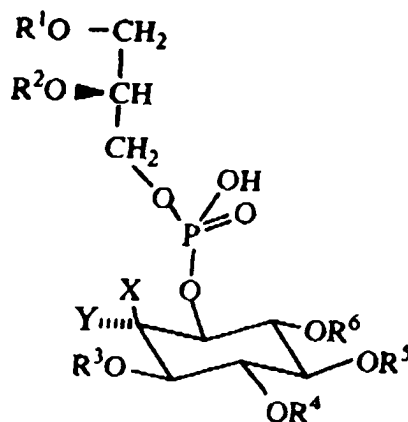
wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

43. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

44. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate.

45. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate.

46. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glyceric ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

and wherein

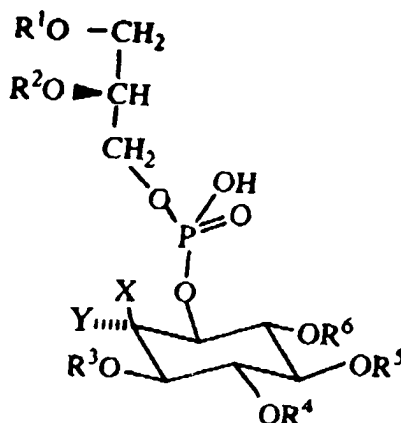
- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ , except when  $R = R' =$  alkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,  
 (d)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,  
 (e)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,

- (f)  $R^6 = H$ ,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

47. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glyceric ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
  - (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $P(=O)(O\text{-protecting group})_2$ , or
  - (c)  $X = Y = F$  or  $(=O)$ ;
- where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

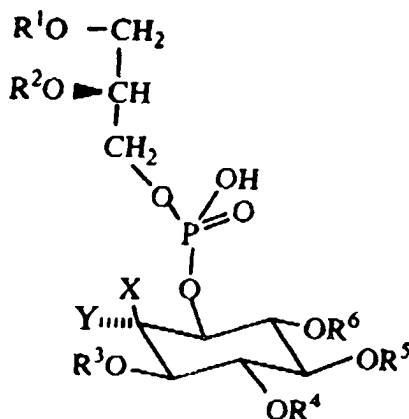
- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R = \text{alkyl}$ , alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' = \text{alkyl}$ , alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,

$\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (f)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (g)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (h)  $R^6 = H$ ,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

48. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
  - (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ ; or
  - (c)  $X = Y = F$  or  $(=O)$ ;
- where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

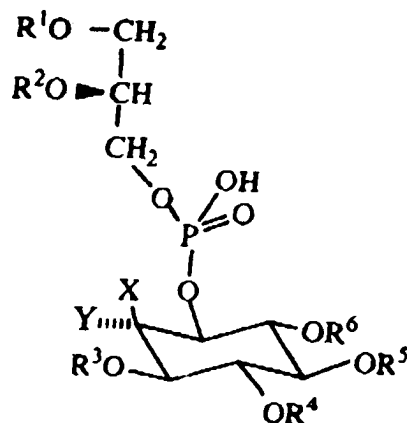
and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R, R' = \text{alkyl or alkenyl}$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (g)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

49. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

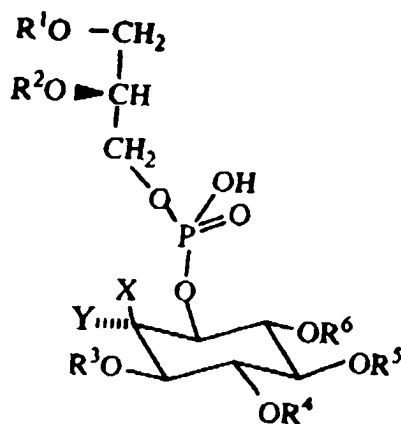
and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R =$  alkyl, alkenyl, alkynyl,  $R' =$   $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R =$   $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ , except when  $R = R' =$  alkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

50. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ , and  $Y = H$ ; or  $X = Y = H$ ; or
  - (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR$ , or  $OP(=O)(OH)_2$ ; or
  - (c)  $X = Y = F$  or  $(=O)$ ;
- where  $R =$  alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-

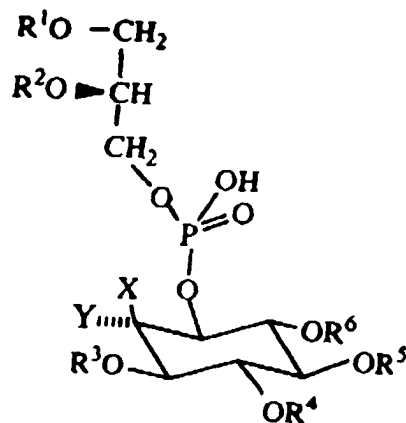
alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (g)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

51. Matched pairs of the 2-modified phosphatidylinositol-phosphates of claim 48 and the corresponding phosphatidylinositol-phosphate structure lacking the 2-modification, wherein  $X=OH$  and  $Y=H$ , or  $X=H$  and  $Y=OH$ .

52. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$



where R = alkyl, alkenyl, alkynyl, R' =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, [alkyl-fluorophor], hydroxylalkyl, or ketoalkyl; or where R' = alkyl, alkenyl, alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, hydroxylalkyl, or ketoalkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

53. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a conjugand suitable for linking to a reporter group, polymer, chromatographic matrix, or gold surface is incorporated in the fattyacyl or inositol residue; wherein said conjugand is selected from the group consisting of  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl, hydroxylalkyl and ketoalkyl, and wherein the amino, substitutedamino, sulfhydryl, carboxyl, hydroxyl and keto functions are free and unsubstituted, or are covalently linked to a reporter group;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**EXHIBIT C**  
**EXPLANATION OF AMENDMENTS**  
**WITH REFERENCE TO SERIAL NO.08/872,222**

The following explanations of the changes in the substitute specification are made with reference to the text of Application Serial No. 08/872,222 as originally filed.

At page 1, line 3 of the text, before "This invention was", the inserted text reads -- The present application claims priority to co-pending application Serial No. 08/872,222, filed June 10, 1997; which claims priority to provisional application Serial No. 60/019,651, filed June 11, 1996. --.

At page 6, line 22 of the text, the deleted text reads "themajor" and the inserted text reads -- the major --.

At page 10, line 23 of the text, in the center of the page, the deleted text reads "methoxybenzyl)-*myo*-inositol" and the inserted text reads -- methoxybenzyl)-*myo/scyllo*-inositol --.

At page 11, lines 5, 11, 13, 19, 23 and bridging lines 25 and 26 of the text, each instance of the deleted text reads "-*myo*-inositol" and each instance of the inserted text reads -- *myo/scyllo*-inositol --.

At page 12, line 10 of the text, the deleted text reads "-*myo*-inositol" and the inserted text reads -- *myo/scyllo*-inositol --.

At page 12, lines 15 and 24 of the text, in the center of the page, each instance of the deleted text reads "-*scyllo*-inositol" and each instance of the inserted text reads -- *myo/scyllo*-inositol --.

At page 12, line 18 of the text, the deleted text reads "*myo*-inositol" and the inserted text reads -- *myo/scyllo*-inositol --.

At page 13, line 2 of the text, the deleted text reads "-*myo*-inositol" and the inserted text reads -- *myo/scyllo*-inositol --.

At page 13, lines 3 and 20 of the text, each instance of the deleted text reads "-*scyllo*-inositol" and each instance of the inserted text reads -- *myo/scyllo*-inositol --.

At page 14, lines 6, 8 and 11 of the text, in the center of the page, each instance of the deleted text reads "-*myo*-inositol" and each instance of the inserted text reads -- *myo/scyllo*-inositol --.

At page 14, line 18 of the text, the deleted text reads "1D-3,6-Di-*O*-benzyl-4,5-di-*O*-cyclohexylidene-1-(*p*-methoxybenzyl)-*myo*-inositol" and the inserted text reads -- 1D-3,6-Di-*O*-benzyl-4,5-*O*-cyclohexylidene-1-(*p*-methoxybenzyl)-*myo*-inositol --.

Exhibit D

Searching 1999-2000...

Results of Search in 1999-2000 db for:

"reporter group" OR "reporter molecule": 790 patents.

Hits 1 through 50 out of 790

Next 50 Hits

Jump to:

Refine Search:

PAT. NO.	Title
1 6,114,517	Methods of modulating tumor necrosis factor .alpha.-induced expression of cell adhesion molecules
2 6,114,513	Thiol-derivatized oligonucleotides
3 6,114,350	Cyanine dyes and synthesis methods thereof
4 6,114,177	Fluorometric assay for measurement of antioxidant activity
5 6,114,160	Compositions and methods for taxol biosynthesis
6 6,114,117	Homogeneous diagnostic assay method utilizing simultaneous target and signal amplification
7 6,113,904	Human glycoprotein
8 6,111,094	Enhanced antisense modulation of ICAM-1
9 6,111,085	Carbamate-derivatized nucleosides and oligonucleosides
10 6,110,747	Compounds and methods for modulating tissue permeability
11 6,110,722	F.sub.0 ATP synthase subunit
12 6,110,693	Methods of assaying receptor activity and constructs useful in such methods
13 6,110,687	Detection of antigens via oligonucleotide antibody conjugates
14 6,110,686	DNA hybridizing to a human cystatin-like protein (CSTIN)
15 6,110,682	Signal enhancement method and kit
16 6,110,677	Oligonucleotide modification, signal amplification, and nucleic acid detection by target-catalyzed product formation
17 6,110,675	Reagents and methods useful for detecting diseases of the prostate
18 6,110,664	Antisense inhibition of G-alpha-S1 expression
19 6,110,630	Efficient activated cyanine dyes
20 6,110,507	Human 3-hydroxyisobutryl-coenzyme A hydrolase
21 6,107,472	Receptor-type tyrosine kinase-like molecules
22 6,107,283	Cardiac glycosides inhibit proliferation of cells bearing FGF receptors

- 23 6,107,092 Antisense modulation of SRA expression
- 24 6,107,091 Antisense inhibition of G-alpha-16 expression
- 25 6,107,039 Assays using base protected table 1
- 26 6,106,844 Immunomodulatory peptides of vespid antigen 5
- 27 6,106,732 Integral blood plasma or serum isolation, metering and transport device
- 28 6,103,877 Tumor suppressor gene, HIC-1
- 29 6,103,874 Human KDEL receptor
- 30 6,103,537 Capillary assays involving separation of free and bound species
- 31 6,103,497 Human S100 proteins
- 32 6,103,483 Molecule involved in binding of sperm to egg surfaces and procedures for use of this molecule to enhance or decrease potential fertility
- 33 6,103,479 Miniaturized cell array methods and apparatus for cell-based screening
- 34 6,103,477 Rho protein
- 35 6,103,474 Hybridization assay signal enhancement
- 36 6,103,469 Human phospholipase A2 protein
- 37 6,103,217 Polymeric assemblies for sensitive colorimetric assays
- 38 6,103,199 Capillary electroflow apparatus and method
- 39 6,100,090 Antisense inhibition of PI3K p85 expression
- 40 6,100,075 Delta 1-pyrroline-5-carboxylate reductase homolog
- 41 6,100,048 Methods and reagents for discovering and using mammalian melanocortin receptor agonists and antagonists to modulate feeding behavior in animals
- 42 6,100,040 Methods and compositions for detection of specific nucleotide sequences
- 43 6,100,037 Human cyclic nucleotide PDEs
- 44 6,100,036 NADH dehydrogenase B17 subunit
- 45 6,100,034 Detection of retroviral subtypes based upon envelope specific sequences
- 46 6,100,027 Nucleic acid probes and amplification oligonucleotides for Neisseria species
- 47 6,100,024 Methods and compositions for nucleic acid detection by target extension and probe amplification
- 48 6,099,803 Advanced active electronic devices for molecular biological analysis and diagnostics
- 49 6,096,725 Methods of using .alpha.Gal oligosaccharides as immune system targeting agents
- 50 6,096,722 Antisense modulation of cell adhesion molecule expression and treatment of cell adhesion molecule-associated diseases

Searching 1999-2000...

Results of Search in 1999-2000 db for:  
 "reporter group" OR "reporter molecule": 790 patents.  
 Hits 51 through 100 out of 790

Prev 50 Hits

Next 50 Hits

Home

Refine Search: "reporter group" OR "reporter molecule"

PAT. NO.	Title
51 6,096,720	Liposomal oligonucleotide compositions
52 6,096,543	Antisense inhibition of human mek1 expression
53 6,096,502	Substrate for detecting UL9 helicase activity
54 6,096,308	Human protein kinase and kinase inhibitors
55 6,093,811	Oligonucleotide modulation of cell adhesion
56 6,093,809	Telomerase
57 6,093,565	Protein phosphatase regulatory subunit
58 6,093,538	Nucleic acid probes to ureaplasma
59 6,093,537	Double receptor polynucleotide assay method
60 6,091,003	Compositions and methods for genetic transformation of pineapple
61 6,090,786	Serine proteases, their activity and their synthetic inhibitors
62 6,090,631	Methods and compositions for screening for presynaptic calcium channel blockers
63 6,090,626	Antisense oligonucleotide modulation of raf gene expression
64 6,090,620	Genes and gene products related to Werner's syndrome
65 6,090,606	Cleavage agents
66 6,090,591	Selective amplification of target polynucleotide sequences
67 6,090,589	Nucleic acid amplification with DNA-dependent RNA polymerase activity of RNA replicases
68 6,090,578	MTS1 gene
69 6,090,577	Disease associated acidic protein
70 6,090,564	SnRNP Sm proteins
71 6,090,543	Cleavage of nucleic acids
72 6,090,390	Diagnostic test for equine arteritis virus mediated disease

9/11/00

- 73 6,090,386 T cell peptides of the CRX JII allergen
- 74 6,090,377 Monocyte activating cytokine
- 75 6,087,489 Antisense oligonucleotide modulation of human thymidylate synthase expression
- 76 6,087,486 Nucleotide sequences encoding vpr receptor protein
- 77 6,087,333 Disease associated acidic protein
- 78 6,087,178 Method for down regulating CD4 expression in a T cell
- 79 6,087,173 Antisense modulation of X-linked inhibitor of apoptosis expression
- 80 6,087,172 Ribozymes targeted to human IL-15 mRNA
- 81 6,087,125 Polynucleotide encoding a novel human nm23-like protein
- 82 6,087,108 RNA editing enzyme
- 83 6,084,102 Covalently linked oligonucleotide minor groove binder conjugates
- 84 6,084,082 Lactam nucleic acids
- 85 6,084,070 Human glutaredoxin .beta.
- 86 6,083,929 Extended type 1 chain glycosphingolipids as tumor-associated antigens
- 87 6,083,923 Liposomal oligonucleotide compositions for modulating RAS gene expression
- 88 6,083,758 Method for screening peptides for metal coordinating properties and fluorescent chemosensors derived therefrom
- 89 6,083,750 Adenovirus vectors
- 90 6,083,724 Recombinant avian interferon-gamma (IFN-.gamma.)
- 91 6,083,706 Inhibitors of leaderless protein export
- 92 6,083,704 Human cytochrome b5
- 93 6,083,689 Sensitive immunoassays utilizing antibody conjugates with replicable DNA templates
- 94 6,080,868 Nitro-substituted non-fluorescent asymmetric cyanine dye compounds
- 95 6,080,848 Human brain associated protein
- 96 6,080,847 Proteins associated with cell proliferation
- 97 6,080,842 Human ATP binding-cassette transport protein
- 98 6,080,841 Human induced tumor protein
- 99 6,080,723 Human actVA-ORF4-like protein
- 100 6,080,558 Polynucleotide encoding human growth regulator protein



US006093538A

# United States Patent [19]

Hogan et al.

[11] Patent Number: 6,093,538

[45] Date of Patent: Jul. 25, 2000

## [54] NUCLEIC ACID PROBES TO UREAPLASMA

[75] Inventors: James J. Hogan, Coronado; Diane L. McAllister, San Diego; Patricia Gordon, Spring Valley; Philip W. Hammond, Tehachapi, all of Calif.

[73] Assignee: Gen-Probe Incorporated, San Diego, Calif.

[21] Appl. No.: 08/109,037

[22] Filed: Aug. 18, 1993

## Related U.S. Application Data

[63] Continuation-in-part of application No. 07/879,686, May 6, 1992, abandoned.

[51] Int. Cl.<sup>7</sup> ..... C12Q 1/68; C07H 21/04

[52] U.S. Cl. .... 435/6; 536/24.32; 536/24.33; 536/23.1

[58] Field of Search ..... 435/6; 536/24.32; 536/24.33, 23.1; 935/878; 514/44

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Primary Examiner—Carla J. Myers  
 Attorney, Agent, or Firm—Brobeck, Phleger & Harrison

## [57] ABSTRACT

Hybridization assay probes are described which are able to distinguish *Ureaplasma* and known strains or serotypes of the species *Ureaplasma urealyticum* found in humans from other related organisms.

107 Claims, No Drawings

## NUCLEIC ACID PROBES TO UREAPLASMA

This application is a continuation-in-part of Kacian et al., entitled "Nucleic Acid Sequence Amplification Method, Composition and Kit," U.S. Ser. No. 07/879,686 filed May 6, 1992, now abandoned hereby incorporated by reference herein.

## FIELD OF THE INVENTION

The invention described and claimed herein relates to the design and use of nucleic acid probes capable of detecting organisms of the genus *Ureaplasma*, and known strains or serotypes of the species *Ureaplasma urealyticum*, in test samples, e.g., from urogenital and endocervical specimens, tissue samples, amniotic and other body fluids, and from cultures.

## BACKGROUND OF THE INVENTION

Two single strands of deoxyribo- ("DNA") or ribo- ("RNA") nucleic acid, formed from nucleotides, (including the bases adenine (A), cytosine (C), thymidine (T), guanine (G), uracil (U), or inosine (I)), may hybridize to form a double-stranded structure held together by hydrogen bonds between pairs of complementary bases. Generally, A is hydrogen bonded to T or U, while G or I are hydrogen bonded to C. Along the chain, classical base pairs AT or AU, TA or UA, GC, or CG are present. Additionally, some mismatched base pairs (e.g., AG, GU) may be present.

Bringing together two single strands of nucleic acid containing sufficient contiguous complementary bases, under conditions which will promote their hybridization, results in double-stranded nucleic acid. Under appropriate conditions, DNA/DNA, RNA/DNA, or RNA/RNA hybrids can form.

A probe is generally a single-stranded nucleic acid sequence complementary to some degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a reporter group moiety such as a radioisotope, a fluorescent or chemiluminescent moiety, or with an enzyme or other ligand which can be used for detection. Background descriptions of the use of nucleic acid hybridization to detect particular nucleic acid sequences are given in Kohne, U.S. Pat. No. 4,851,330 issued Jul. 25, 1989, and by Hogan et al., International Patent Application No. PCT/US87/03009, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," both references hereby incorporated by reference herein. Hogan et al., supra, describe methods for determining the presence of a non-viral organism or a group of non-viral organisms in a sample (e.g., sputum, urine, blood and tissue sections, food, soil and water).

The genera *Ureaplasma* and *Mycoplasma* are prokaryotes and comprise the taxonomic Mollicutes class. Mollicutes lack a bacterial cell wall and have a small genome size. They are considered one of the smallest of the free-living microorganisms. *Ureaplasma* are unique among Mollicutes because of their characteristic ability to metabolize urea. There are fourteen known serotypes of *U. urealyticum* (Stemke and Robertson, *Diagn. Microbiol. Infect. Dis.* 31: 311 (1985)). The fourteen serotypes can be divided into at least two subspecies ("biotypes") based upon restriction fragment length polymorphism ("RFLP") of *U. urealyticum* genomic DNA (Harasawa et al., *Abstract S30-6 UIMS Meeting*, Osaka Japan (1990), and Robertson et al., *J. Clin. Microbiol.* 31: 824 (1993)), or based upon rRNA sequences (Hammond et al., *Abstract D17. Session 60, American Society for Microbiology General Meeting*, (1991)).

*U. urealyticum* is commonly found in the human urogenital tract but has been implicated in a wide spectrum of pathologies. Several studies have implicated *U. urealyticum* as a possible etiologic agent in diseases affecting adult males, fetuses and infants. Brunner et al., *Yale J. Biol. Med.* 56: 545 (1983), identified *U. urealyticum* as the etiologic agent responsible for nongonococcal urethritis (NGU) in approximately 30 percent of adult males tested who had NGU. Cassell et al., *Pediatr. Infect. Dis.* 5: S247 (1986), implicated *U. urealyticum* as a possible cause of chorioamnionitis, which could in turn adversely affect the outcome of pregnancy and the health of neonates. Stagno et al., *Pediatrics* 68: 322 (1981), found *U. urealyticum* in 21% of infants with pneumonia and found the association of *U. urealyticum* with pneumonia to be "statistically significant." Waites et al., *Lancet* 8575: 17 (1988), found *U. urealyticum* in 8 percent of the cerebrospinal fluid specimens taken from a high-risk population of newborn infants (100 predominantly pre-term infants). According to these investigators *U. urealyticum* was the most common organism isolated of those sought. *U. urealyticum* has also been implicated in a number of other pathogenic states including septic arthritis (Lee et al., *Arthritis and Rheumatism* 35: 43 (1992)).

Standard microbiological techniques generally identify *U. urealyticum* by observing the hydrolysis of urea. These techniques usually involve inoculating both a complex broth medium and an agar medium containing urea and other nutrients with a freshly obtained specimen (Brunner et al., supra).

References concerning detection of *Ureaplasma* include the following: Roberts et al., *Israel J. Med. Sci.*, 23: 618 (1987), describe the use of whole chromosomal DNA probes for detection of *Ureaplasma* in genital specimens; Ohse and Göbel, *Israel J. Med. Sci.* 23: 352 (1987) describe hybridization of *U. urealyticum* rRNA genes to cloned DNA of the *E. coli* rRNA operon; Göbel and Stanbridge ("Detection of *Mycoplasma* by DNA Hybridization", EPO application number 86304919.3, publication number 0 250 662) mention biological probes for detecting *Mycoplasmas* or prokaryotes in general, or specific *Mycoplasma* and eubacterial species; Gonzales et al. (*American Society for Microbiology Annual Meeting* 1991, *Abstract D-16*) mentions a method to detect *Ureaplasma* using a DNA probe directed to rRNA; Lee et al., supra, and Willoughby et al., *Infection and Immunity* 59: 2463 (1991), describe a procedure for detecting the *U. urealyticum* urease gene utilizing PCR; Deng et al., *PCR Methods and Applications* 1: 202 (1992), suggest that PCR-RFLP techniques should be capable of detecting Mollicutes; Brogan et al., *Molecular and Cellular Probes* 6: 411 (1992), describe the amplification of a 186 base pair genomic *U. urealyticum* DNA fragment; Robertson et al., supra, describe a technique involving the polymerase chain reaction using biotype specific primers to 16S rRNA gene sequences to distinguish the two *U. urealyticum* biotypes.

## SUMMARY OF THE INVENTION

The featured invention discloses and claims novel oligonucleotide probes which are either targeted to a specific *Ureaplasma* nucleic acid target sequence or consist essentially of a specified nucleic acid sequence. The probes function by hybridizing to target *U. urealyticum* rRNA and/or rRNA gene sequences under stringent hybridization assay conditions. Thus, the probes can distinguish the genus *Ureaplasma*, including clinically significant *U. urealyticum* serotypes, from their known closest phylogenetic neighbors (*Mycoplasma*) and from other microorganism inhabitants of the human urogenital tract. Accordingly, the probes may be



used in an assay to detect and/or quantitate *Ureaplasma* and *U. urealyticum* organisms.

Species of *Mycoplasma* found in humans include *M. genitalium*, *M. pneumoniae* and *M. hominis*. *M. pneumoniae* appears to be the most closely related *Mycoplasma* to *U. urealyticum*. *M. genitalium* is very similar in nucleic acid sequence to *M. pneumoniae* and has been isolated from the human genital tract. *M. hominis* is the most commonly isolated *Mycoplasma* from the genital tract.

Thus, in a first aspect, the invention described herein features hybridization assay probes able to selectively hybridize to a *Ureaplasma* target nucleic acid sequence. A *Ureaplasma* target nucleic acid sequence is a nucleic acid sequence present in *Ureaplasma*, preferably *U. urealyticum* nucleic acid, or a sequence complementary thereto. Preferably, the target nucleic acid sequence is not present in closely related *Mycoplasma* (e.g., *M. pneumoniae*). Sequences complementary to a target sequence may be generated by target amplification techniques such as polymerase chain reaction (PCR) or transcription mediated amplification (e.g., Kacian and Fultz, entitled "Nucleic Acid Amplification Methods", EPO application number 90307503.4; and Kacian et al., supra entitled "Nucleic Acid Sequence Amplification Method, Composition and Kit."

The featured probes can detect *U. urealyticum* and distinguish the genus *Ureaplasma* and known strains or serotypes of *U. urealyticum* found in humans from other bacteria including the phylogenetic closely related *M. pneumoniae*.

A hybridization assay probe is comprised of an oligonucleotide having a nucleic acid sequence sufficiently complementary to hybridize, under stringent hybridization assay conditions, to a 5S, 16S, or 23S rRNA, or to the corresponding ribosomal DNA ("rDNA") nucleic acid sequence, or to a nucleic acid sequence complementary thereto, of *U. urealyticum*. Stringent hybridization assay conditions, refer to conditions wherein a specific probe hybridizes with target nucleic acid (e.g., rRNA of *Ureaplasma*) and not another nucleic acid present in the test sample from either other microorganisms (e.g., *Mycoplasma pneumoniae*) or humans. The probes are preferably 10 to 100 nucleotides in length.

By "probe" is meant to exclude naturally occurring nucleic acids. Purified oligonucleotide probes may be produced by techniques known in the art such as chemical synthesis and by in vitro or in vivo expression from recombinant nucleic acid molecules, e.g., retroviral vectors.

An oligonucleotide is made of nucleotide subunits covalently joined together. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof such as O-methyl ribose. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages, or by non-nucleotide moieties that do not prevent hybridization of the oligonucleotide. Modified linkages include those linkages in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage, or methylphosphonate linkage. When used as a hybridization assay probe, the oligonucleotide preferably contains a reporter group such as acridinium ester or a radioisotope to help identify hybridization of the probe to its target sequence.

In a related aspect, the invention described herein features hybridization assay probes able to selectively hybridize to a *U. urealyticum* nucleic acid target sequence present on either biotype 1 or biotype 2. The claimed target sequence is present in only one of the biotypes. Thus, an oligonucleotide probe directed to either biotype 1 or biotype 2 target site can distinguish between the biotypes.

In another related aspect, hybridization assay probes having a specific nucleic acid sequences complementary to rRNA or rDNA of *Ureaplasma*, are described. The probes are useful for detecting and/or quantitating *Ureaplasma* which may be present. These probes are complementary to a region of rRNA or rDNA which varies between *Ureaplasma* and *Mycoplasma*. Specific probes able to hybridize to *Ureaplasma* nucleic acid and distinguish *Ureaplasma* from *Mycoplasma*, comprise, consist essentially of, or consist of the sequences (written 5' to 3'):

(SEQ ID NO: 2) ACCTCTCAGT ACAGCTACGC G  
(SEQ ID NO: 5) CATTTCCCTAT CTAGCGTTT CTTCCC  
(SEQ ID NO: 8) CGTTAAGCAT CTAGATTAA TAC-  
CAAACTT ACAAACCCG

(SEQ ID NO: 9) CCTACTACAC TCTAGGTTTA  
CAGTTTTTGA TACAGCTAGA

(SEQ ID NO: 11) GTCAGTGATA GTCCAAGTTG GC  
(SEQ ID NO: 14) CGTTCGAGCC GACATTTAAT GAT-  
GATCG

(SEQ ID NO: 17) GCGTCGCAAT AGATGTCAAA  
CCTAG

(SEQ ID NO: 20) CGATTTTGCA GCAGTTTGTA  
TTAGCCATTG

(SEQ ID NO: 22) GCTATTTTCG GCTCTAGAGT GCT-  
TGACTTC TGTGTTCCGG ATG

(SEQ ID NO: 23) CGGCTCTAGA GTGCTTGACT TCT-  
GTGTTTCG

(SEQ ID NO: 26) GGATGGGAAC AGGTATTTCC  
ACTCTGATAT GATCAC

(SEQ ID NO: 29) CAGTAATCTA ATTCTCATTG GACT-  
GAGTTT CCTCATTCG and RNA equivalents thereto  
(SEQ ID NOS: 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61,  
and 109), oligonucleotides complementary thereto (SEQ  
ID NOS: 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 and 110),  
and RNA equivalents to the oligonucleotides complemen-  
tary thereto (SEQ ID NOS: 33, 36, 39, 42, 45, 48, 51, 54,  
57, 60, 63, 111). Preferably, helper probe are used to  
facilitate the hybridization of the assay probe to its target  
nucleic acid sequence.

The phrases "consists essentially of" or "consisting essentially of" mean that the probe is provided as an oligonucleotide which hybridizes under stringent hybridization assay conditions to a target nucleic acid sequence of a particular organism and preferably does not hybridize with *Mycoplasma* described herein. A hybridization probe may be linked to other nucleic acids which do not affect hybridization. Generally, it is preferred that the probe be between 10 and 100 (most preferably between 15 and 50) nucleotides in length. Additionally, the probe may be provided in a vector.

For the listed probes, two sets of stringent hybridization assay conditions were used. One set comprised hybridization at 60° C. for one hour in a solution containing 0.095 M lithium succinate pH 5, 0.31 M lithium lauryl sulfate, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 1.5 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetraacetic acid (EGTA). After the one hour, hybrids were separated from unhybridized probe by binding to magnetic amine microspheres in a solution containing 0.76 M sodium borate pH 7.5, 6% Triton at 60° C. for ten minutes and washed once in a solution containing 80 mM sodium borate pH 10.4 at room temperature.

Another set of stringent hybridization assay conditions was comprised of hybridization in 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60° C. for 15 minutes, followed by the addition of 300 µl of 0.6 M sodium borate pH 8.5, 1% Triton X-100 at 60° C. for 5-7 minutes. Additional sets of

stringent hybridization conditions can be determined based upon techniques known in the art and the present disclosure.

In another aspect, specific probes able to distinguish between different biotypes are described. Specific probes able to hybridize to a nucleic acid sequence present in only one *Ureaplasma* biotype comprise, consist essentially of, or consist of the sequences (written 5' to 3'):

SEQ ID NO. 121: CAACACCGAC TCGTTCGAGC

SEQ ID NO. 122: CAACACCGAC CCATTTCGG and RNA equivalents thereto (SEQ ID NOs: 126 and 127), oligonucleotides complementary thereto (SEQ ID NOs: 131 and 132), and RNA equivalents to the oligonucleotides complementary thereto (SEQ ID NOs: 136 and 137). Preferably, a helper probe is used to facilitate the hybridization of the assay probe to its target nucleic acid sequence.

In another aspect, specific helper probe oligonucleotide sequences have been determined. Helper probes are used to facilitate the rate of hybridization of a hybridization assay probe to its target nucleic acid as described by Hogan and Milliman, U.S. Pat. No. 5,030,557 entitled "Means and Method for Enhancing Nucleic Acid Hybridization," issued Jul. 9, 1991 and hereby incorporated by reference herein. Helper probes featured herein include: SEQ ID NOs. 1, 3, 4, 6, 7, 8, 9, 10, 12, 13, 15, 16, 18, 19, 21, 24, 25, 26, 27, 28, 30, 123, 124, 125; RNA equivalents thereto, SEQ ID NOs. 37, 40, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, 118, 128, 129, 130; oligonucleotides complementary thereto, SEQ ID NOs. 38, 41, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107, 110, 113, 116, 119, 133, 134, 135; and RNA equivalents to the oligonucleotides complementary thereto, SEQ ID Nos. 39, 42, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 102, 105, 108, 111, 114, 117, 120, 138, 139, 140.

Some oligonucleotide probes can be used as an assay probe or a helper probe (e.g., SEQ ID Nos. 8, 9, and 26, RNA equivalents thereto, SEQ ID Nos. 37, 40, and 109, oligonucleotides complementary thereto, 38, 41, and 110, and RNA equivalents to the oligonucleotides complementary thereto 39, 42, and 111).

In another related aspect, the invention features compositions comprising a nucleic acid hybrid between a hybridization assay probe and a nucleic acid sequence substantially complementary thereto (probe:target). "Substantially complementary" means there is sufficient complementarity between the nucleic acids such that the hybrid is stable under stringent hybridization conditions. One use of the formed hybrid is to detect the presence of a target sequence. For example, acridinium ester ("AE") present in hybrids is resistant to hydrolysis in alkali solution whereas acridinium ester present in single-stranded nucleic acid is hydrolyzed in alkali solution (Arnold et al., entitled "Homogeneous Protection Assay," EPO application number 88308767.8, publication number 309230, hereby incorporated by reference herein). Thus, binding of AE-labeled probe to target can be detected, after hydrolysis of the unbound AE-labeled probe, by measuring chemiluminescence of acridinium ester remaining in the nucleic acid hybrid.

In other related aspects, methods are described for detecting *Ureaplasma urealyticum* and distinguishing *Ureaplasma urealyticum* from Mycoplasma such as *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*; distinguishing between *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2; and detecting the presence of a *Ureaplasma urealyticum* nucleic acid sequence. These methods can be used on test samples obtained from human specimens.

The probes of this invention offer a rapid, non-subjective method of identifying and quantitating the presence of specific rRNA sequences unique to the genus *Ureaplasma* and all strains of *U. urealyticum* in a test sample.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have identified preferred target sequences present in the rRNA or rDNA of *U. urealyticum* and designed specific oligonucleotide probes to these sequences and their complements which can be used to identify *Ureaplasma*. The probes can detect the genus *Ureaplasma* including *U. urealyticum* serotypes and distinguish them from their known and presumably most closely related taxonomic or phylogenetic neighbors. Probes are also described which distinguish *U. urealyticum* biotype 1 and biotype 2. Also described are methods using the featured probes or target sites.

In a preferred embodiment, the nucleic acid hybridization assay probes can distinguish *U. urealyticum* from *M. genitalium*, *M. pneumoniae*, or *M. hominis*. In another preferred embodiment, the nucleic acid hybridization probes can distinguish *U. urealyticum* from *M. orale*, *M. fermentans*, *M. capricolum*, *M. lipophilum*, and *M. salivarium*. These Mycoplasma have been isolated from humans.

Prokaryotic organisms (excluding viruses) contain rRNA genes encoding 5S rRNA, 16S rRNA and 23S rRNA. Using methods known to those skilled in the art, partial or full rRNA sequences of *U. urealyticum* and Mycoplasma were obtained. These sequences were aligned based on regions of sequence homology. Sequence variations were then identified from the aligned sequences and used as target sequences for hybridization assay probes.

### Obtaining rRNA Sequences

Sequence information was obtained experimentally and from published information (see, Weisburg et al., *J. Bacteriol.* 171: 6455 (1989); and Rogers et al., *Proc. Natl. Acad. Sci., U.S.A.*, 82: 1160 (1985)). Experimental information was obtained by isolating and sequencing the ribonucleic acid from various organisms using sequence standard techniques known in the art. Nucleic acids were obtained using an oligonucleotide primer complementary to a conserved region of rRNA and extending the primer using reverse transcriptase. Nucleic acid sequences were then derived using the method of dideoxynucleotide chain termination. (e.g., Lane et al., *Proc. Natl. Acad. Sci. U.S.A.*, 82: 6955 (1985)).

### Probe Design And Hybridization Conditions

To facilitate the identification of nucleic acid sequences to be used as probes, the nucleotide sequences from different organisms were first aligned to maximize homology. Within the rRNA molecule there is a close relationship between secondary structure and function. This imposes restrictions on evolutionary changes in the primary sequence so that the secondary structure is maintained. For example, if a base is changed on one side of a helix, a compensating change is made on the other side to preserve the complementarity (this is referred to as co-variance). This allows two very different sequences to be aligned based on the conserved primary sequence and also on the conserved secondary structure elements. Potential target sequences for the hybridization probes were identified by noting variations in the homology of the aligned sequences.

The sequence evolution at each of the variable regions is mostly divergent. Because of the divergence, more distant

phylogenetic relatives of *U. urealyticum* show greater variability to *U. urealyticum* at the variable region than phylogenetically closer relatives. We observed sufficient variation between *U. urealyticum* and strains of *Mycoplasma* found in the same sample to design several useful probes and identify preferred target sites.

Selective hybridization of probe to target can be accomplished by choosing the appropriate hybridization assay conditions and proper probe design. The stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay and washing conditions so that hybrids will only form between highly complementary sequences. Manipulation of one or more of the different assay conditions determines the exact sensitivity and specificity of a particular probe. The following guidelines are useful for designing probes and determining stringent hybridization assay conditions.

Probes should be designed to have an appropriate melting temperature ( $T_m$ ). The appropriate  $T_m$  can be obtained by varying the probe length and nucleotide composition (percentage of G+C versus A+T). The probe length and nucleotide composition should preferably be chosen to correspond to a  $T_m$  about 2–10° C. higher than the temperature at which the final assay will be performed.

In general, the optimal hybridization temperature for oligonucleotide probes of about 10–50 bases in length is approximately 5° C. below the melting temperature for a given duplex. Incubation at temperatures below the optimum temperature may allow mismatched base sequences to hybridize and can therefore decrease specificity. The longer the probe, the more hydrogen bonding between base pairs and, in general, the higher the  $T_m$ . Increasing the percentage of G and C also increases the  $T_m$  because G-C base pairs exhibit additional hydrogen bonding and therefore greater thermal stability than A-T base pairs.

The preferred method to determine  $T_m$  measures hybridization using a Hybridization Protection Assay (HPA) according to Arnold et al., supra entitled "Homogeneous Protection Assay."  $T_m$  can be measured using HPA in the following manner. A probe:target hybrid is formed in a lithium succinate buffered solution (0.1 M lithium succinate buffer, pH 5.0, 2 mM EDTA, 2 mM EGTA, 10% (w/v) lithium lauryl sulfate) using an excess amount of target. Aliquots of the hybrid are then diluted in the lithium succinate buffered solution and incubated for five minutes at various temperatures starting below that of the anticipated  $T_m$  (typically 55° C.) and increasing in 2–5° C. increments. This solution is then diluted with a mild alkaline borate buffer (0.15 M sodium tetraborate, pH 7.6, 5% (v/v) Triton X-100) and incubated at a lower temperature (for example 50° C.) for ten minutes. Under these conditions the acridinium ester attached to a single-stranded probe is hydrolyzed while the acridinium ester attached to hybridized probe is relatively protected from hydrolysis. Thus, the amount of acridinium ester remaining is proportional to the amount of hybrid and can be measured by the chemiluminescence produced from the acridinium ester upon the addition of hydrogen peroxide followed by alkali. Chemiluminescence can be measured in a luminometer (e.g., the Gen-Probe LEADER I or LEADER 50). The resulting data is plotted as percent of maximum signal (usually from the lowest temperature) versus temperature. The  $T_m$  is defined as the temperature at which 50% of the maximum signal remains. In addition to the method above,  $T_m$  may be determined by isotopic methods well known to those skilled in the art (e.g., Hogan et al., supra).

It should be noted that the  $T_m$  for a given hybrid varies depending on the hybridization solution used. Factors such

as the salt concentration, detergents, and other solutes can affect hybrid stability during thermal denaturation (J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning*, ch. 11 (2d ed. 1989)). Conditions such as ionic strength and incubation temperature under which a probe will be used to hybridize to target should be taken into account in constructing a probe. Thermal stability of hybrids increases as the ionic strength of the reaction mixture increases. On the other hand, chemical reagents which disrupt hydrogen bonds, such as formamide, urea, dimethyl sulfoxide and alcohols, can greatly reduce the thermal stability of the hybrids.

To ensure specificity of probe for target, it is desirable to have probes which hybridize only under conditions of high stringency. Under conditions of high stringency only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands to form a hybrid. Stringency is chosen to maximize the difference in stability between the hybrid formed with the target and other nucleic acid sequences.

Proper specificity may be achieved by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by constructing the probe to contain as many destabilizing mismatches to nontarget sequences as possible. Whether a probe sequence is useful to detect only a specific type of organism depends largely on the thermal stability difference between probe:target hybrids versus probe:non-target hybrids. In designing probes, the differences in these  $T_m$  values should be as large as possible (preferably 2° C.–5° C. or more).

The length of the target nucleic acid sequence, and accordingly the length of the probe sequence, can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly homologous base sequence will generally determine hybrid stability. Oligonucleotide probes of different lengths and base composition may be used. Preferably, oligonucleotide probes are between 10 to 100 and, more preferably, between 15 to 50 bases in length.

Regions of rRNA known to form strong internal structures inhibitory to hybridization are less preferred target regions. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of two single strands of complementary nucleic acid to form a hydrogen-bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in an intramolecular or intermolecular hybrid it will be less able to participate in the formation of a new intermolecular probe:target hybrid. In the case of rRNA, the molecule is known to form very stable intramolecular hybrids. By designing a probe so that a substantial portion of the targeted sequence is single-stranded, the rate and extent of hybridization between probe and target may be greatly increased.

An rDNA target occurs naturally in a double-stranded form as does the product of the polymerase chain reaction (PCR). These double-stranded targets are naturally inhibitory to hybridization with a probe and require denaturation prior to hybridization. Appropriate denaturation and hybridization conditions are known in the art (e.g., E. M. Southern, *J. Mol. Biol.* 98: 503 (1975)).

### Probe Synthesis

Once a presumptive unique target sequence has been identified, a complementary oligonucleotide probe is selected and synthesized. Defined oligonucleotide probes may be produced by any of several well-known methods, including automated solid-phase chemical synthesis using cyanoethylphosphoramidite precursors (Barone et al., *Nucleic Acids Research* 12: 4051 (1984)), and as described in J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning*, ch. 11 (2d ed. 1989). Following synthesis and purification of a particular oligonucleotide probe, several different procedures may be utilized to determine the acceptability of the probe in terms of size and purity. One such procedure is polyacrylamide gel electrophoresis. Another such procedure is High Pressure Liquid Chromatography ("HPLC"). These procedures are well known to those skilled in the art.

Once synthesized, selected oligonucleotide probes may be labeled with a reporter group by any of several well-known methods (e.g., supra, J. Sambrook et al.). Useful labels include radioisotopes and non-radioactive reporting groups. Isotopic labels include  $^3\text{H}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{125}\text{I}$ ,  $^{57}\text{Co}$  and  $^{14}\text{C}$ . Isotopic labels can be introduced into the oligonucleotide by techniques known in the art such as nick translation, end labeling, second strand synthesis, the use of reverse transcription, and by chemical methods. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the particular radioisotope used for labeling.

Non-isotopic materials can also be used for labeling and may be introduced internally into the nucleic acid sequence or at the end of the nucleic acid sequence. Modified nucleotides may be incorporated enzymatically or chemically. Chemical modifications of the probe may be performed during or after synthesis of the probe, for example, through the use of non-nucleotide linker groups as described by Arnold et al., entitled "Non-Nucleotide Linking Reagents for Nucleotide Probes," EPO application number 88308766.0, publication number 313219, hereby incorporated by reference herein. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands.

Preferably, the probes are labeled with an acridinium ester. Acridinium ester labeling may be performed as described by Arnold et al., U.S. Pat. No. 5,185,439 entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes" issued Feb. 9, 1993 and hereby incorporated by reference herein.

### Helper Probes

The rate of nucleic acid hybridization of an assay probe with its target nucleic acid is enhanced by the use of "Helper Probes" as disclosed in Hogan and Milliman, U.S. Pat. No. 5,030,557 and hereby incorporated by reference herein. Helper probes are selected to hybridize to nucleic acid sequences located near the region targeted by the assay probe. Hybridization of the helper probe alters the secondary and tertiary structure and thereby renders the targeted area of the nucleic acid more accessible for the detection probe. Helper probes to be used with the assay probes described herein include oligonucleotides having the following nucleotide sequences (written 5' to 3'):

(SEQ ID NO: 1) TCATTGACTT GGTGAGCCAT TACCT-CAC

(SEQ ID NO: 3) GCCGTGTCTC AGTCCCATTTG TGGCT-GTTCT

(SEQ ID NO: 4) ATATAAAAGA ACTTTACAAT CTATAAGACC TTCATCGTTC ACGCGGC

(SEQ ID NO: 6) GGCACATAGT TAGCCGATAC TTAT-TCAAAT GGTACAGTCA AA

(SEQ ID NO: 7) CCTGCGCTCG TTTTACGCCC AGTAAATCCG GATAACGC

(SEQ ID NO: 8) CGTTAAGCAT CTAGATTTAA TAC-CAAACCT ACAAAACCG

(SEQ ID NO: 9) CCTACTACAC TCTAGGTTTA CAGTTTTTGA TACAGCTAGA

(SEQ ID NO: 10) GCCTTCGCCA CCGGTGTTCT TCCATATATC TA

(SEQ ID NO: 12) CTAATCCTAT TTGCTCCCCA CACTTTCGAG CCTAAGC

(SEQ ID NO: 13) TTTACGGTGT GGACTACTAG GGTAT

(SEQ ID NO: 15) GCGTTAGCTA CAACACCGAC T

(SEQ ID NO: 16) GTAAGGTTCT ACGTGATTG TCAAATTAAG CAACATGCTC CACCAC

(SEQ ID NO: 18) CGACAACCAT GCACCACCTG TCATATTGTT AACCTCAAC

(SEQ ID NO: 19) TAGCAGCTTT GCAGCCCTAG ATATAAGGGG CATGATG

(SEQ ID NO: 21) CGAATTGCAG CCCTCTATCC GAACTGAGAC TAACTTTTTTTC TG

(SEQ ID NO: 24) GGAACAGGTA TTTCCACTCT GATATGATCA CTAC,

(SEQ ID NO: 25) GCGTAGCGAT GACCTATTTT ACT-TGC

(SEQ ID NO: 26) GGATGGGAAC AGGTATTTCC ACTCTGATAT GATCAC,

(SEQ ID NO: 27) GCGTAGCGAT GACCTATTTT ACT-TGCGCTA TTTT

(SEQ ID NO: 28) GAGATCAACG GATTAAAGCC TCT-TATCAGC TACCCGTTGC TTATCGCAGA TTAG-CACG

(SEQ ID NO: 30) CACTTCACCA GGTATCGCTC TGT-TAAACTA TGAATTCATT TATA

(SEQ ID NO: 123) CGACATTTAA TGATGATCGT TTACGGTGTG GAC,

(SEQ ID NO: 124) GCCGACATTT AATGATGATC GTT-TACGGTG TGGAC,

(SEQ ID NO: 125) CCCAGGCACA TCATTTAATG CGTTAGCTA, RNA equivalents thereto, SEQ ID NOs. 37, 40, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, 118, 128, 129, 130; oligonucleotides complementary thereto, SEQ ID NOs. 38, 41, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107, 110, 113, 116, 119, 133, 134, 135; and RNA equivalents to the oligonucleotides complementary thereto, SEQ ID Nos. 39, 42, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 98, 102, 105, 108, 111, 114, 117, 120, 138, 139, 140.

Preferably, the following hybridization assay probe and helper probe combinations are used:

	Hybridization probe	Helper probes
SEQ ID NOs:	2	1 and 3
SEQ ID NOs:	5	4 and 6
SEQ ID NOs:	8	7 and 9
SEQ ID NOs:	9	8 and 10
SEQ ID NOs:	11	10 and 12
SEQ ID NOs:	14	13 and 15
SEQ ID NOs:	17	16 and 18
SEQ ID NOs:	20	19 and 21
SEQ ID NOs:	22	24 and 25
SEQ ID NOs:	23	26 and 27

-continued

	Hybridization probe	Helper probes
SEQ ID NOs:	29	28 and 30
SEQ ID NOs:	121	123 and 125
SEQ ID NOs:	122	124 and 125

## EXAMPLES

Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention.

Probes specific for *Ureaplasma* were identified by sequencing with primers complementary to the 16S and 23S rRNAs of *U. urealyticum* T-960 (CX-8), or from published 5S sequences. The nucleic acid sequence from phylogenetically near neighbors, including *M. genitalium*, *M. pneumoniae*, *M. iowae*, *M. muris*, *M. pirum* and *M. gallisepticum*, were used as comparisons with the nucleic acid sequence from *U. urealyticum* to determine variable regions.

The following hybridization assay probe sequences are featured in the examples described below::

(SEQ ID NO: 2) ACCTCTCAGT ACAGCTACGC G  
 (SEQ ID NO: 5) CATTTCCTAT CTAGCGTTT CTCC  
 (SEQ ID NO: 8) CGTTAAGCAT CTAGATTAA TAC-  
 CAAACTT ACAAAACCG  
 (SEQ ID NO: 9) CCTACTACAC TCTAGGTTTA  
 CAGTTTTTGA TACAGCTAGA  
 (SEQ ID NO: 11) GTCAGTGATA GTCCAAGTTG GC  
 (SEQ ID NO: 14) CGTTCGAGCC GACATTAAAT GAT-  
 GATCG  
 (SEQ ID NO: 17) GCGTCGCAAT AGATGTCAAA  
 CCTAG  
 (SEQ ID NO: 20) CGATTTTGCA GCAGTTTGTA  
 TTAGCCATTG  
 (SEQ ID NO: 22) GCTATTTTCG GCTCTAGAGT GCT-  
 TGACTTC TGTGTTCCGGG ATG  
 (SEQ ID NO: 23) CGGCTCTAGA GTGCTTGACT TCT-  
 GTGTTCCG  
 (SEQ ID NO: 29) CAGTAATCTA ATTCTCATTA GACT-  
 GAGTTT CCTCATTCG  
 (SEQ ID NO: 59) CGAACACAGA AGTCAAGCAC  
 TCTAGAGCCG,  
 (SEQ ID NO: 110) GTGATCATAT CAGAGTGGA  
 ATACCTGTTT CCATCC,  
 (SEQ ID NO: 121) CAACACCGAC TCGTTCGAGC, and  
 (SEQ ID NO: 122) CAACACCGAC CCAATTCGG.

The probes were synthesized with a non-nucleotide linker as described by Arnold et al. supra, "Non-Nucleotide Linking Reagents For Nucleotide Probes," then labeled with a chemiluminescent acridinium ester as described by Arnold et al., supra, U.S. Pat. No. 5,185,439. The reactivity and specificity of the probes for *U. urealyticum* were demonstrated using a hybridization and separation format (Example 1, Tables 1-4) or a homogeneous assay format (Examples 2 and 3, Tables 5 and 6; Example 4, Tables 7 and 8). These procedures are described by Arnold et al., supra, "Homogeneous Protection Assay"; Arnold et al., "Polycationic Supports and Nucleic Acid Purification, Separation and Hybridization" EPO application number 88301839.2, publication number 0 281 390 (hereby incorporated by reference herein); and Arnold et al., *Clin. Chem.*, 35:1588 (1989) (hereby incorporated by reference herein).

Results are given in relative light units (RLU). Probes were hybridized to a cell lysate or RNA purified according

to J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning* (2d ed. 1989). Alternatively, lysates, especially of Mycobacteria, Gram positive organisms, or yeasts, could be obtained utilizing a method described by Murphy et al., "Method for Releasing RNA and DNA from Cells," EPO application number 87303641.2, publication number 288618, hereby incorporated by reference herein. The following examples describe hybridization assay probes targeted to *U. urealyticum* rRNA sequences, or the corresponding gene, and their use in a hybridization assay.

## Example 1

This example illustrates the ability of a mixture containing acridinium ester-labeled probes targeted to *Ureaplasma* 16S rRNA to detect various *Ureaplasma* strains but not other microorganisms. The mixture contained assay probes having SEQ ID NOs. 2, 5, 8, 9, 11, 14, 17 and 20, and the corresponding unlabeled "Helper Probes" (as described above).

Table 1 presents data using these probes with an excess of RNA released from liquid broth cultures containing  $10^5$ - $10^8$  organisms. An equal volume of cell lysate and hybridization solution containing 0.19 M lithium succinate pH 5, 0.62 M lithium lauryl sulfate, 3 mM ethylenediaminetetraacetic acid (EDTA), 3 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetraacetic acid (EGTA) were mixed and incubated at 60° C. for one hour. Hybrids were then bound to magnetic amine microspheres (Advanced Magnetics, Inc., Cambridge, Mass.) in a solution containing 0.76 M sodium borate pH 7.5, 6% Triton and washed once in a solution containing 80 mM sodium borate pH 10.4. The chemiluminescence associated with the particles, from the hybridized acridinium ester-labeled probes, was measured in a luminometer equipped with automatic injection of 0.1% hydrogen peroxide in 1 mM nitric acid, followed by injection of a 1N sodium hydroxide solution. RLU from a hybridization reaction containing 1 ng of non-target RNA was subtracted from the values shown. The data in Table 1 show that the probes hybridize to known strains or serotypes of *U. urealyticum* found in humans as well as to *U. cati*, *U. diversum* and *U. gallorale* of animal origin.

Table 2 shows that the probes distinguish *Ureaplasma* from several closely related *Mycoplasma*, *Acholeplasma*, or *Spiroplasma* species. A net RLU value greater than 300 RLU was considered a positive reaction. An all-bacteria/yeast probe mixture was used as a control to demonstrate the presence of bacterial nucleic acid (data not shown). Hogan et al., supra, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," gives examples of suitable all-bacteria/yeast probe mixtures. The all-bacteria probe used in the examples described herein is a derivative of all-bacteria probe No. 7 described by Hogan et al., (the all-bacteria probe used in the examples described herein is shifted so that it is four nucleotides shorter on the 5' end but 5 bases longer on the 3' end probe than the Hogan probe No. 7). The yeast probe is a derivative of fungal probe No. 1 described in Hogan et al.

Table 3 shows that the assay probe mixture distinguishes *Ureaplasma* from members of a panel of urogenital microbes. The all-bacteria/yeast probe mixture was also used as a control in this experiment.

Table 4 shows that the assay probes distinguish *Ureaplasma* from twenty-seven bacterial genera representing a phylogenetic cross section of microorganisms. Again, the all-bacteria/yeast probe mixture was used as a control in this experiment.

TABLE 1

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH UREAPLASMA STRAINS AND SEROTYPES			
ATCC NO.	ORGANISM/ STRAIN	SEROTYPE	NET RLU <sup>a</sup>
	<i>U. urealyticum</i> strain		
27813	7	1	634,146
27618	T-960(CX8)	8	592,533
27814	23	2	775,013
27816	58	4	758,427
27619	K510(CX4)	—	906,488
27815	27	3	703,288
27817	354	5	474,113
27818	Pi	6	769,951
27819	Co	7	780,741
29557	K71-21	4	876,253
29558	K42-35	4	933,227
29559	K12-19	4/8	892,978
33175	Vancouver	9	576,453
33695	K2	11	875,684
33696	U24	12	863,070
33697	U26	14	677,350
33698	U38	13	749,523
33699	Western	10	862,237
49228	<i>U. cati</i>	—	467,562
43321	<i>U. diversum</i>	—	772,938
43346	<i>U. gallorale</i>	—	1,161,922

<sup>a</sup>Chemiluminescence was measured in a Gen-Probe LEADER I luminometer and data are expressed in net Relative Light Units (signal minus the negative control containing 1 ng non-Ureaplasma rRNA).

TABLE 2

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH OTHER MOLICUTES			
ORGANISM	ATCC NO.	EXPERIMENT NO.	PROBE MIX NET RLU
<i>Mycoplasma fermentans</i> <sup>a</sup>	15474	1	15
<i>Mycoplasma gallisepticum</i> <sup>a</sup>	19610	1	30
<i>Mycoplasma genitalium</i> <sup>a</sup>	33530	1	31
<i>Mycoplasma hominis</i> <sup>a</sup>	23114	1	38
<i>Mycoplasma iowae</i> <sup>a</sup>	33552	1	81
<i>Mycoplasma muris</i> <sup>a</sup>	33757	1	17
<i>Mycoplasma pirum</i> <sup>a</sup>	25960	1	26
<i>Mycoplasma pneumoniae</i> <sup>a</sup>	15531	1	62
<i>Spiroplasma mirum</i> <sup>a</sup>	29335	1	105
<i>Spiroplasma</i> sp. MQ-1 <sup>a</sup>	33825	1	66
<i>Acholeplasma laidlawii</i> <sup>c</sup>	29804	2	180
<i>Mycoplasma arthritidis</i> <sup>c</sup>	35943	2	14
<i>Mycoplasma buccale</i> <sup>c</sup>	23636	2	58
<i>Mycoplasma orale</i> <sup>c</sup>	23714	2	-18
<i>Mycoplasma primatum</i> <sup>c</sup>	15497	2	-29
<i>Mycoplasma salivarium</i> <sup>c</sup>	14277	2	11
<i>Ureaplasma urealyticum</i> <sup>b</sup>	27618	2	938

<sup>a</sup>0.10 ng purified RNA.

<sup>b</sup>0.01 ng purified RNA.

<sup>c</sup>Whole cell lysates from 10<sup>7</sup>-10<sup>8</sup> organisms.

TABLE 3

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH UROGENITAL MICROBES			
ORGANISM <sup>a</sup>	ATCC NO.	UREAPLASMA 16S PROBES NET RLU	ALL- BACTERIA/ YEAST PROBES NET RLU
10 <i>Bacteroides fragilis</i>	23745	43	605,178
<i>Bacteroides ureolyticus</i>	43605	37	112,716
<i>Candida albicans</i>	18804	26	13,380
<i>Chlamydia trachomatis</i>	VR-878	-1	76,109
<i>Clostridium perfringens</i>	13124	-8	419,044
15 <i>Eikenella corrodens</i>	23834	-13	812,060
<i>Gardnerella vaginalis</i>	14018	11	55,694
<i>Haemophilus influenzae</i>	9795	6	1,203,162
<i>Lactobacillus acidophilus</i>	4356	-10	424,616
<i>Listeria monocytogenes</i>	35152	-8	33,993
<i>Mycobacterium smegmatis</i>	14468	1	14,392
20 <i>Neisseria gonorrhoeae</i>	19424	122	147,963
<i>Peptostreptococcus anaerobius</i>	27337	-8	290,081
<i>Staphylococcus aureus</i>	12598	29	16,256
<i>Staphylococcus epidermidis</i>	12228	66	4,519
<i>Torulopsis glabrata</i>	2001	0	646,442

<sup>a</sup>Whole cell lysates were tested at a concentration of 10<sup>7</sup> cells per reaction.

TABLE 4

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH A PHYLOGENETIC PANEL			
ORGANISM <sup>a</sup>	ATCC NO.	UREAPLASMA 16S PROBES NET RLU	ALL- BACTERIA/ YEAST PROBES NET RLU
30 <i>Ureaplasma urealyticum</i>	27618	1,170	ND
<i>Alcaligenes faecalis</i>	8750	-6	751,053
<i>Bacillus subtilis</i>	6051	14	19,523
40 <i>Campylobacter jejuni</i>	33560	2	1,079,901
<i>Chromobacterium violaceum</i>	29094	10	1,026,462
<i>Citrobacter freundii</i>	6750	2	758,996
<i>Actinomyces pyogenes</i>	19411	12	148,548
<i>Corynebacterium xerosis</i>	373	38	2,091
<i>Deinococcus radiodurans</i>	35073	-4	78,908
45 <i>Derris gummosa</i>	15994	20	753,002
<i>Enterobacter aerogenes</i>	13048	8	967,109
<i>Enterobacter cloacae</i>	10699	5	1,078,720
<i>Enterococcus avium</i>	14025	32	10,594
<i>Enterococcus faecalis</i>	19433	42	32,000
<i>Erwinia herbicola</i>	33243	9	821,862
<i>Escherichia coli</i>	10798	66	959,572
50 <i>Klebsiella pneumoniae</i>	23357	12	1,326,216
<i>Legionella pneumophila</i>	33152	34	869,560
<i>Micrococcus luteus</i>	9341	50	6,256
<i>Plesiomonas shigelloides</i>	14029	17	837,909
<i>Proteus mirabilis</i>	25933	17	927,223
<i>Pseudomonas aeruginosa</i>	10145	5	1,285,353
55 <i>Pseudomonas fluorescens</i>	13525	10	1,318,299
<i>Rhodospirillum rubrum</i>	11170	25	563,898
<i>Streptococcus agalactiae</i>	13813	21	204,717
<i>Streptococcus bovis</i>	33317	7	402,823
<i>Vibrio parahaemolyticus</i>	17802	7	1,138,932
60 <i>Yersinia enterocolitica</i>	9610	7	1,136,326

<sup>a</sup>Whole cell lysates were tested at a concentration of 10<sup>7</sup> cells per reaction. The Ureaplasma sample contained 0.01 ng of *Ureaplasma urealyticum* rRNA.  
ND = not done.

## Example 2

Hybridization of an acridinium ester-labeled probe, targeted to a 23S rRNA *U. urealyticum* region, to *U. urealyti-*

cum and other bacteria was evaluated. Lysate (L) or purified RNA was hybridized to probe SEQ ID NO. 29 and helper probes SEQ ID NOs. 28 and 30 in 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60° C. for 15 minutes, followed by addition of 300 µl of 0.6 M sodium borate pH 8.5, 1% Triton X-100 at 60° C. for 5-7 minutes. Samples were read in a luminometer as described in Example 1. The *Ureaplasma* sample contained 1 µg of *U. urealyticum* rRNA.

As shown in Table 5, probes targeted to 23S rRNA *U. urealyticum* readily distinguish *U. urealyticum* from other organisms including *Mycoplasma*. The data in this table is reported in RLU without subtracting background and Negative control values. Values greater than about 20,000 to 30,000 RLU were considered positive results in this assay.

TABLE 5

HYBRIDIZATION OF UREAPLASMA-SPECIFIC 23S rRNA PROBES TO OTHER MOLLICUTES AND <i>E. COLI</i>		
ORGANISM	ATCC NO.	23S PROBE RLU
<i>Mycoplasma arthritis</i> (L)	35943	746
<i>Mycoplasma buccale</i> (L)	23636	565
<i>Mycoplasma fermentans</i> (L)	15474	948
<i>Mycoplasma iowae</i> (L)	33552	4,241
<i>Mycoplasma muris</i> (L)	33757	4,346
<i>Mycoplasma pirum</i> (L)	25960	596
<i>Mycoplasma primatum</i> (L)	15497	709
<i>Mycoplasma salivarium</i> (L)	14277	629
<i>Spiroplasma</i> sp. MQ-1 (L)	33825	737
<i>Acholeplasma laidlawii</i>	29804	1,052
<i>Mycoplasma gallisepticum</i>	19610	432
<i>Mycoplasma genitalium</i>	33530	4,503
<i>Mycoplasma hominis</i>	23114	450
<i>Mycoplasma orale</i>	23714	945
<i>Mycoplasma pneumoniae</i>	15531	4,073
<i>Spiroplasma mirum</i>	29335	431
<i>Escherichia coli</i>	10798	772
<i>Ureaplasma urealyticum</i>	27618	1,307,260

## Example 3

Acridinium ester-labeled probe SEQ ID NOs. 22 or 23 targeted to 5S rRNA was hybridized to an excess of RNA released from cells in the form of cell lysate or purified as described above and assayed as described in Example 2. Probe SEQ ID NO. 22 was hybridized in the presence of helper probes SEQ ID NOs. 24 and 25; probe SEQ ID NO. 23 was hybridized in the presence of helper probes SEQ ID NOs. 26 and 27.

As shown in Table 6, the probes targeted to *Ureaplasma urealyticum* 5S rRNA were able to distinguish this organism from other Mollicutes.

TABLE 6

HYBRIDIZATION OF UREAPLASMA 5S rRNA PROBES TO MOLLICUTES			
ORGANISM	ATCC NO.	PROBE SEQ ID NO. 22 RLU	PROBE SEQ ID NO. 23 RLU
<i>Mycoplasma arginini</i>	23838	1,332	3,655
<i>Mycoplasma arthritis</i> *	35943	1,382	3,957
<i>Mycoplasma bovigenitalium</i> *	19852	1,395	4,864
<i>Mycoplasma bovis</i> *	25523	1,280	4,885
<i>Mycoplasma buccale</i> *	23636	1,332	5,762
<i>Mycoplasma californicum</i> *	33461	1,466	6,218

TABLE 6-continued

HYBRIDIZATION OF UREAPLASMA 5S rRNA  
PROBES TO MOLLICUTES

ORGANISM	ATCC NO.	PROBE SEQ ID NO. 22 RLU	PROBE SEQ ID NO. 23 RLU
<i>Mycoplasma capricolum</i> *	23205	1,496	5,064
<i>Mycoplasma faucium</i> *	25293	1,466	6,218
<i>Mycoplasma fermentans</i> *	15474	2,017	10,572
<i>Mycoplasma gallisepticum</i>	19610	1,355	5,657
<i>Mycoplasma genitalium</i>	33530	1,233	4,721
<i>Mycoplasma muris</i> *	33757	5,640	12,462
<i>Mycoplasma iowae</i> *	33552	2,537	6,498
<i>Mycoplasma pirum</i> *	25960	1,674	7,354
<i>Mycoplasma lipophyllum</i> *	27790	1,559	5,103
<i>Mycoplasma neurolyticum</i> *	19988	1,482	5,861
<i>Mycoplasma orale</i>	23714	1,697	4,362
<i>Mycoplasma pneumoniae</i> *	15531	2,129	7,514
<i>Mycoplasma primatum</i> *	15497	1,530	4,787
<i>Mycoplasma salivarium</i> *	23064	1,662	4,676
<i>Spiroplasma mirum</i> *	29335	2,815	7,227
<i>Ureaplasma urealyticum</i> *	27815	895,233	676,817
<i>Ureaplasma urealyticum</i> *	27619	1,679,357	1,449,564

\*Whole cell lysates were tested at a concentration of 10<sup>7</sup> cells per reaction.

## Example 4

This example describes probes which can distinguish biotype 1 from biotype 2. In the course of probe development it was observed that one probe gave signals substantially lower for biotype 1 lysates than biotype 2 lysates. This suggested sequence variability in the probe region. To identify probe sequences targeted to a particular biotype several strains of *Ureaplasma urealyticum* were analyzed. Using the sequence information, biotype specific probes SEQ ID NOs. 121 and 122 were synthesized and labeled with acridinium ester. The probes were hybridized to rRNA from 18 strains of *Ureaplasma urealyticum* as described in Example 2 and the data is presented in Table 7. The signal obtained with the all-bacteria/yeast probe mix provides a quantitative indication of the amount of rRNA in each sample. The biotype 1 probe reacted only with biotype 1 strains; the biotype 2 probe reacted only with biotype 2 strains.

A similar experiment was performed to investigate the specificity of the biotype probes against 18 closely related *Mycoplasma* species and two *Spiroplasma* species. Results shown in Table 8 are the net RLU (i.e., the RLU from sample tested minus the RLU from a negative control sample). As seen in Table 8, the biotype-specific *Ureaplasma urealyticum* probes reacted only with their respective specific biotype strains and did not cross-react with any of the other closely related organisms.

TABLE 7

## HYBRIDIZATION OF BIOTYPE PROBES

PROBE, NET RLU				
<i>U. urealyticum</i> ATCC NO.	Biotype	All-Bacteria/ Yeast	Biotype 1	Biotype 2
27813	1	141,390	10,830	913
27815	1	95,249	60,145	130
27818	1	87,785	30,091	101
33697	1	83,584	77,891	130
27618	2	120,574	120	117,078

TABLE 7-continued

HYBRIDIZATION OF BIOTYPE PROBES				
<i>U. urealyticum</i> ATCC NO.	Biotype	PROBE, NET RLU		
		All-Bacteria/ Yeast	Biotype 1	Biotype 2
27814	2	142,847	5	128,002
27816	2	112,627	89	148,618
27619	2	159,929	958	180,885
27817	2	69,874	108	69,151
27819	2	101,053	61	146,858
29557	2	113,125	143	128,480
29558	2	133,822	55	104,791
29559	2	92,546	644	150,724
33175	2	60,896	93	95,811
33695	2	122,517	106	143,790
33696	2	115,043	183	134,746
33698	2	112,323	3	125,216
33699	2	98,076	125	127,981

TABLE 8

SPECIFICITY OF BIOTYPE PROBES				
ORGANISM	ATCC NO.	PROBE, NET RLU		
		All-Bacteria/ Yeast	Biotype 1	Biotype 2
<i>M. arginini</i>	23838	33,238	230	-184
<i>M. arthritidis</i>	35943	141,240	82	82
<i>M. bovis genitalium</i>	19852	9,543	17	26
<i>M. bovis</i>	25523	70,824	-111	96
<i>M. buccale</i>	23636	15,210	-143	31
<i>M. californicum</i>	33461	113,936	77	26
<i>M. capricolum</i>	23205	50,103	-97	95
<i>M. faucium</i>	25293	61,263	84	21
<i>M. fermentans</i>	15474	34,324	2	-16
<i>M. gallisepticum</i>	19610	62,053	-119	31
<i>M. genitalium</i>	33530	104,629	215	-5
<i>M. pirum</i>	25960	59,082	106	93
<i>M. neurolyticum</i>	19988	17,383	95	72
<i>M. orale</i>	23715	29,103	22	113
<i>M. pneumoniae</i>	15531	34,329	-161	-94
<i>M. primate</i>	15497	40,730	-18	23
<i>M. salivarium</i>	23064	66,612	-80	-60
<i>M. hominis</i>	23114	46,680	-58	36
<i>Sp. mirum</i>	29335	53,887	-19	34
<i>Sp. MQ-1</i>	33825	35,178	-51	24
<i>U. urealy. bio. 1</i>	27815	62,491	42,268	163
<i>U. urealy. bio. 2</i>	27619	108,404	125	136,790

## Example 5

This example illustrates the use of assay probes for *Ureaplasma* of the same sense as the target nucleic acid to detect the products of target nucleic acid amplification. *Ureaplasma urealyticum* rRNA was amplified by incubation at about 37° C. in 100  $\mu$ L of a solution comprising 0.3  $\mu$ M of a promoter-primer (SEQ. ID. No. 141), 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 17.5 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 2 mM spermidine trihydrochloride, 6.5 mM rATP, 2.5 mM rCTP, 6.5 mM rGTP, 2.5 mM rUTP, 0.2 mM DATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 600 U MuMLV reverse transcriptase and 400 U T7 RNA polymerase (Kacian et al., supra, entitled "Nucleic Acid Sequence Amplification Method, Composition, and Kit"). The reaction was monitored by removing aliquots at various time points between 15 minutes and 4 hours and assaying for the product using two 5S rRNA probes of the same sense as the target rRNA (SEQ. ID. Nos. 59, 110) and helper probes (SEQ. ID. Nos. 104, 107) using conditions described in Example 2.

TABLE 9

Time of Incubation	RLU	
	1 fmol target	0.1 fmol target
15 min	5,389	307
30 min	10,360	778
60 min	40,622	5,588
120 min	144,851	13,051
180 min	192,618	16,249
240 min	203,393	20,745

The data shown in Table 9 demonstrates the ability of assay probes targeted to nucleic acid sequences of the opposite sense as the rRNA of the organism to detect the product from a target amplification procedure. As the amplification time increased, more target sequence was produced resulting in increased signal from probe detection.

The data shown in the various examples described above confirm that the novel probes herein described and claimed are capable of distinguishing *Ureaplasma* from its known nearest phylogenetic neighbors. The data also demonstrates that probes have been designed which can be used to distinguish *Ureaplasma* biotypes from each other and from nearest known phylogenetic neighbors. Furthermore, complementary oligonucleotide probes, i.e., those having the same sense as the target, are utilized to detect the products of target amplification procedures now being utilized to increase the detection sensitivity of assays for organisms.

Other embodiments are within the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 141

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear



-continued

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:  
TCATTGACTT GGTGAGCCAT TACCTCAC 28

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:  
ACCTCTCAGT ACAGCTACGC G 21

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 3:  
GCCGTGTCTC AGTCCCATTG TGGCTGTTCT 30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 47  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:  
ATATAAAGA ACTTTACAAT CTATAAGACC TTCATCGTTC ACGCGGC 47

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:  
CATTTCCTAT CTTAGCGTTT CTTCCTC 26

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 6:  
GGCACATAGT TAGCCGATAC TTATTCAAAT GGTACAGTCA AA 42

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

-continued

CCTGCGCTCG TTTTACGCCC AGTAAATCCG GATAACGC

38

## (2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 39           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGTTAAGCAT CTAGATTAA TACCAACTT ACAAAACCG

39

## (2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 40           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCTACTACAC TCTAGTTTA CAGTTTTGA TACAGCTAGA

40

## (2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 32           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCCTTCGCCA CCGGTGTTCT TCCATATATC TA

32

## (2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 22           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTCAGTGATA GTCCAAGTTG GC

22

## (2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 37           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTAATCCTAT TTGCTCCCA CACTTCGAG CCTAAGC

37

## (2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
- |                   |              |
|-------------------|--------------|
| (A) LENGTH:       | 25           |
| (B) TYPE:         | nucleic acid |
| (C) STRANDEDNESS: | single       |
| (D) TOPOLOGY:     | linear       |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

-continued

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TTTACGGTGT GGACTACTAG GGTAT 25

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CGTTCGAGCC GACATTTAAT GATGATCG 28

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCGTTAGCTA CAACACCGAC T 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTAAGGTTCT ACGTGTATTG TCAAATTAAG CAACATGCTC CACCAC 46

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCGTCGCAAT AGATGTCAAA CCTAG 25

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CGACAACCAT GCACCACCTG TCATATTGTT AACCTCAAC 39

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG 37

-continued

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CGATTTTGCA GCAGTTTGTA TTAGCCATTG

30

## (2) INFORMATION FOR SEQ ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CGAATTGCAG CCCTCTATCC GAACTGAGAC TAACTTTTTC TG

42

## (2) INFORMATION FOR SEQ ID NO: 22:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCTATTTTCG GCTCTAGAGT GCTTGACTTC TGTGTTCCGGG ATG

43

## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CGGCTCTAGA GTGCTTGACT TCTGTGTTTCG

30

## (2) INFORMATION FOR SEQ ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGAACAGGTA TTTCCACTCT GATATGATCA CTAC

34

## (2) INFORMATION FOR SEQ ID NO: 25:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GCGTAGCGAT GACCTATTTT ACTTGC

26

-continued

## (2) INFORMATION FOR SEQ ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GGATGGGAAC AGGTATTTC ACTCTGATAT GATCAC

36

## (2) INFORMATION FOR SEQ ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CGTAGCGAT GACCTATTTT ACTTGCCTA TTTT

34

## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAGATCAACG GATTAAAGCC TCTTATCAGC TACCCGTTGC TTATCGCAGA TTAGCAGC

58

## (2) INFORMATION FOR SEQ ID NO: 29:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CAGTAATCTA ATTCTCATT GACTGAGTTT CCTCATTCG

39

## (2) INFORMATION FOR SEQ ID NO: 30:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CACTTCACCA GGTATCGCTC TGTTAAACTA TGAATTCATT TATA

44

## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ACCUCUCAGU ACAGCUACGC G

21

-continued

## (2) INFORMATION FOR SEQ ID NO: 32:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CGCGTAGCTG TACTGAGAGG T

21

## (2) INFORMATION FOR SEQ ID NO: 33:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CGCGUAGCUG UACUGAGAGG U

21

## (2) INFORMATION FOR SEQ ID NO: 34:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAUUUCCUAU CUUAGCGUUU CUUCCC

26

## (2) INFORMATION FOR SEQ ID NO: 35:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGGAAGAAAC GCTAAGATAG GAAATG

26

## (2) INFORMATION FOR SEQ ID NO: 36:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GGGAAGAAAC GCUAAGAUAG GAA AUG

26

## (2) INFORMATION FOR SEQ ID NO: 37:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CGUUAAGCAU CUAGAUUUA UACCAAACUU ACAAAACCG

39

## (2) INFORMATION FOR SEQ ID NO: 38:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	39
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CGGGTTTGTA AGTTTGGTAT TAAATCTAGA TGCTTAACG 39

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	39
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CGGGUUUGUA AGUUDGGUUA UAAAUCUAGA UGCUUAACG 39

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	40
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA 40

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	40
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

TCTAGCTGTA TCAAAACTG TAAACCTAGA GTGTAGTAGG 40

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	40
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG 40

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GUCAGUGAUA GUCCAAGUUG GC 22

(2) INFORMATION FOR SEQ ID NO: 44:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GCCAACTTGG ACTATCACTG AC 22

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GCCAACUUGG ACUAUCACUG AC 22

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

CGUUCGAGCC GACAUUUAU GAUGAUCG 28

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CGATCATCAT TAAATGTCGG CTCGAACG 28

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CGAUCAUCAU UAAAGUCGG CUCGAACG 28

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	25
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GCGUGCAAU AGAUGUCAA CCUAG 25

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:



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(A) LENGTH: 25  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CTAGGTTTGA CATCTATTGC GACGC

25

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CUAGGUUUGA CAUCUAUUGC GACGC

25

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

CGAUUUUGCA GCAGUUUGUA UUAGCCAUUG

30

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CAATGGCTAA TACAAACTGC TGCAAAATCG

30

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

CAAUGGCUAA UACAAACUGC UGCAAAAUGC

30

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GCUAUUUUGC GCUCUAGAGU GCUUGACUUC UGUGUUCGGG AUG

43

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43

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(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 56:  
 CATCCCGAAC ACAGAAGTCA AGCACTCTAG AGCCGAAAAT AGC 43

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 43  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 57:  
 CAUCCCGAAC ACAGAAGUCA AGCACUCUAG AGCCGAAAAU AGC 43

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 58:  
 CGGCUCUAGA GUGCUUGACU UCUGUGUUCG 30

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 59:  
 CGAACACAGA AGTCAAGCAC TCTAGAGCCG 30

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 60:  
 CGAACACAGA AGUCAAGCAC UCUAGAGCCG 30

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 61:  
 CAGUAAUCUA AUUCUCAUUA GACUGAGUUU CCUCAUUCG 39

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 39  
 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 62:  
CGAATGAGGA AACTCAGTCT AATGAGAATT AGATTACTG 39

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 63:  
CGAAGGAGGA AACUCAGUCU AAUGAGAAU AGAUUACUG 39

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 64:  
UCAUUGACUU GGUGAGCCAU UACCUCAC 28

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 65:  
GTGAGGTAAT GGCTCACCAA GTCAATGA 28

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 66:  
GUGAGGUAAU GGCUCACCAA GUCAAUGA 28

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 67:  
GCCGUGUCUC AGUCCCAUUG UGGCUGUUCU 30

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

AGAACAGCCA CAATGGGACT GAGACACGGC 30

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	30
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

AGAACAGCCA CAAUGGGACU GAGACACGGC 30

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	47
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

AUAUAAAAGA ACUUUACAAU CUUAAGACC UUCAUCGUUC ACGCGGC 47

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	47
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

GCCGCGTGAA CGATGAAGGT CTTATAGATT GTAAAGTTCT TTTATAT 47

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	47
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

GCCGCGUGAA CGAUGAAGGU CUUAUAGAUU GUAAAGUUCU UUUUAUUAU 47

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	42
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

GGCACAUAGU UAGCCGAUAC UUAUUCAAAU GGUACAGUCA AA 42

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	42
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 74:  
TTTGACTGTA CCATTGAAT AAGTATCGGC TAACTATGTG CC 42

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 75:  
UUUGACUGUA CCAUUUGAAU AAGUAUCGGC UAACUAUGUG CC 42

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 76:  
CCUGCGCUCG UUUUACGCC AGUAAUCCG GAUAACGC 38

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 77:  
GCGTTATCCG GATTACTGG GCGTAAAACG AGCGCAGG 38

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 38  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 78:  
GCGUUAUCCG GAUUUACUGG GCGUAAAACG AGCGCAGG 38

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 79:  
GCCUUCGCCA CCGGUGUUCU UCCAUUAUUC UA 32

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 80:  
TAGATATATG GAAGAACACC GGTGGCGAAG GC 32

(2) INFORMATION FOR SEQ ID NO: 81:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 32  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 81:  
UAGAUUAUUG GAAGAACACC GGUGGCGAAG GC 32

(2) INFORMATION FOR SEQ ID NO: 82:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 37  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 82:  
CUAAUCCUUAU UUGCUCUCCCA CACUUUCGAG CCUAAGC 37

(2) INFORMATION FOR SEQ ID NO: 83:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 37  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 83:  
GCTTAGGCTC GAAAGTGTGG GGAGCAAATA GGATTAG 37

(2) INFORMATION FOR SEQ ID NO: 84:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 37  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 84:  
GCUUAGGCUC GAAAGUGUGG GGAGCAAUA GGAUUG 37

(2) INFORMATION FOR SEQ ID NO: 85:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 25  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 85:  
UUUACGGUGU GGACUACUAG GGUAU 25

(2) INFORMATION FOR SEQ ID NO: 86:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 25  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear  
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

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ATACCCTAGT AGTCCACACC GTAAA

25

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

AUACCCUAGU AGUCCACACC GUAAA

25

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

GCGUUAGCUA CAACACCGAC U

21

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

AGTCGGTGTI GTAGCTAACG C

21

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

AGUCGGUGUU GUACCUAAG C

21

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

GUAAGGUUCU ACGUGUAUUG UCAAAUUAAG CAACAUGCUC CACCAC

46

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

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GTGGTGGAGC ATGTGCTTA ATTTGACAAT ACACGTAGAA CCTTAC

46

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

GUGGUGGAGC AUGUUGCUUA AUUUGACAAU ACACGUAGAA CCUUAU

46

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

CGACAACCAU GCACCACCUG UCAUAUUGUU AACCUCAAC

39

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

GTTGAGGTTA ACAATATGAC AGGTGGTGCA TGGTTGTCG

39

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

GUUGAGGUUA ACAADAUGAC AGGUGGUGCA UGGUUGUCG

39

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

UAGCACGUUU GCAGCCCUG AUUAUAGGGG CAUGAUG

37

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

CATCATGCCC CTTATATCTA GGGCTGCAAA CGTGCTA

37



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## (2) INFORMATION FOR SEQ ID NO: 99:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

CAUCAUGCCC CUUAUAUCUA GGGCUGCAAA CGUGCUA

37

## (2) INFORMATION FOR SEQ ID NO: 100:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

CGAAUUGCAG CCCUCUAUCC GAACUGAGAC UAACUUUUUC UG

42

## (2) INFORMATION FOR SEQ ID NO: 101:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

CAGAAAAAGT TAGTCTCAGT TCGGATAGAG GGCTGCAATT CG

42

## (2) INFORMATION FOR SEQ ID NO: 102:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAUU CG

42

## (2) INFORMATION FOR SEQ ID NO: 103:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

GGAACAGGUA UUUCCACUCU GAUAUGAUCU CUAC

34

## (2) INFORMATION FOR SEQ ID NO: 104:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

GTAGTGATCA TATCAGAGTG GAAATACCTG TTCC

34

-continued

## (2) INFORMATION FOR SEQ ID NO: 105:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

GUAGUGAUC AUACAGAGUG GAAAUACCUG UUC

34

## (2) INFORMATION FOR SEQ ID NO: 106:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

GCGUAGCGAU GACCUAUUUU ACUUGC

26

## (2) INFORMATION FOR SEQ ID NO: 107:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

GCAAGTAAAA TAGGTCATCG CTACGC

26

## (2) INFORMATION FOR SEQ ID NO: 108:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

GCAAGUAAAA UAGGUCAUCG CUACGC

26

## (2) INFORMATION FOR SEQ ID NO: 109:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GGAUGGGAAC AGGUUUUCC ACUCUGAUAU GAUCAC

36

## (2) INFORMATION FOR SEQ ID NO: 110:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

GTGATCATAT CAGACTGGAA ATACCTGTTC CCATCC

36

-continued

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- (2) INFORMATION FOR SEQ ID NO: 111:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 36
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 111:
- GUGAUCAUU CAGAGUGGAA AUACCUUUC CCAUCC 36
- (2) INFORMATION FOR SEQ ID NO: 112:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 112:
- GCGUAGCGAU GACCUUUUU ACUUGCGUA UUUU 34
- (2) INFORMATION FOR SEQ ID NO: 113:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 113:
- AAAATAGCGC AAGTAAATA GGTCATCGCT ACGC 34
- (2) INFORMATION FOR SEQ ID NO: 114:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 114:
- AAAAUAGCGC AAGUAAAUA GGUCAUCGU ACGC 34
- (2) INFORMATION FOR SEQ ID NO: 115:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 115:
- GAGAUCAACG GAUUAAGCC UCUUAUCAGC UACCCGUUC UUAUCGCAGA UUAGCAGC 58
- (2) INFORMATION FOR SEQ ID NO: 116:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 116:
- CGTGCTAATC TGCGATAAGC AACGGGTAGC TGATAAGAGG CTTTAATCCG TTGATCTC 58
- (2) INFORMATION FOR SEQ ID NO: 117:

-continued

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	58
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

CGUGCUAAUC UGCGAUAGC AACGGGUAGC UGAUAAGAGG CUUUAUCCG UUGAUCUC 58

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	44
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

CACUUCACCA GGUADCGCUC UGUUAAACUA UGAAUOCAU UUAU 44

(2) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	44
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

TATAAATGAA TTCATAGTTT AACAGAGCGA TACCTGGTGA AGTG 44

(2) INFORMATION FOR SEQ ID NO: 120:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	44
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

UAUAAAUGAA UUCAUAGUUU AACAGAGCGA UACCUGGUGA AGUG 44

(2) INFORMATION FOR SEQ ID NO: 121:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	20
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

CAACACCGAC TCGTTCGAGC 20

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	18
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

CAACACCGAC CCATTTCGG 18

(2) INFORMATION FOR SEQ ID NO: 123:

-continued

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	33
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

CGACATTTAA TGATGATCGT TTACGGTGTG GAC 33

(2) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	35
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

GCCGACATTT AATGATGATC GTTTACGGTG TGGAC 35

(2) INFORMATION FOR SEQ ID NO: 125:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	29
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

CCCAGGCACA TCATTTAATG CGTTAGCTA 29

(2) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	20
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

CAACACCGAC UCGUUCGAGC 20

(2) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	18
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

CAACACCGAC CCAUUCGG 18

(2) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	33
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

CGACAUUUAA UGAUGAUCGU UUACGGUGUG GAC 33

(2) INFORMATION FOR SEQ ID NO: 129:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 35  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

GCCGACAUUU AAUGAUGAUC GUUUACGGUG UGGAC 35

(2) INFORMATION FOR SEQ ID NO: 130:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

CCCAGGCACA UCAUUUAAUG CGUUAGCUA 29

(2) INFORMATION FOR SEQ ID NO: 131:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

GCTCGAACGA GTCGGTGTG 20

(2) INFORMATION FOR SEQ ID NO: 132:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

CCGAATGGGT CGGTGTG 18

(2) INFORMATION FOR SEQ ID NO: 133:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 133:

GTCCACACCG TAAACGATCA TCATTAAATG TCG 33

(2) INFORMATION FOR SEQ ID NO: 134:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

GTCCACACCG TAAACGATCA TCATTAAATG TCGGC 35

(2) INFORMATION FOR SEQ ID NO: 135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29

-continued

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(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 135:  
 TAGCTAACGC ATTAAATGAT GTGCCTGGG 29

(2) INFORMATION FOR SEQ ID NO: 136:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 136:  
 GCUCGAACGA GUCGGUGUUG 20

(2) INFORMATION FOR SEQ ID NO: 137:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 18  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 137:  
 CCGAAUGGU CGGUGUUG 18

(2) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 33  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 138:  
 GUCCACACCG UAAACGAUCA UCAUUAUAUG UCG 33

(2) INFORMATION FOR SEQ ID NO: 139:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 35  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 139:  
 GUCCACACCG UAAACGAUCA UCAUUAUAUG UCGGC 35

(2) INFORMATION FOR SEQ ID NO: 140:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 140:  
 UAGCUAACGC AUUAAUAUGAU GUGCCUGGG 29

(2) INFORMATION FOR SEQ ID NO: 141:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 53  
 (B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

AATTTAATAC GACTCACTAT AGGGAGAGCG TAGCGATGAC CTATTTTACT TGC

53

What is claimed is:

1. A hybridization assay probe 10 to 100 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* target nucleic acid sequence to form a detectable probe:target hybrid with said *Ureaplasma urealyticum* target nucleic acid sequence under stringent hybridization assay conditions, wherein said *Ureaplasma urealyticum* target nucleic acid sequence is selected from the group consisting of

SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
UAAAUUCUAGA UGCUUAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
UAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG GC,  
SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUUU CCUCAUUCG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
AAUGAGAAUU AGAUUACUG,  
SEQ ID NO: 109: GGAUGGGAAC AGGUUUUCC  
ACUCUGAUUAU GAUCAC, and  
SEQ ID NO: 111: GUGAUCUAU CAGAGUGGAA  
AUACCUGUC CCAUCC;

wherein under said stringent hybridization assay conditions said hybridization assay probe does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma hominis*.

2. The hybridization assay probe of claim 1, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium* and *Mycoplasma pneumoniae*.

3. The hybridization assay probe of claim 1, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*.

4. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.

5. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.

6. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.

7. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is

10

selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.

8. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.

9. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.

10. A hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 21 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,  
SEQ ID NO: 8: CGTTAAGCAT CTAGATTAA TAC-  
CAAACCT ACAAACCCG,  
SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA  
CAGTTTTTGA TACAGCTAGA,  
SEQ ID NO: 11: CTCAGTGATA GTCCAAGTTG GC,  
SEQ ID NO: 20: CGATTTTGCA GCAGTTTGTA  
TTAGCCATTG,  
SEQ ID NO: 26: GGATGGGAAC AGGTATTTC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 29: CAGTAATCTA ATTCTCATTG GACT-  
GAGTTT CCTCATTCG,  
SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,  
SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
UAAAUUCUAGA UGCUUAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAACCTG  
TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
UAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG GC,  
SEQ ID NO: 44: GCCAACTTGG ACTATCACTG AC,  
SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,  
SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
UUAGCCAUUG,  
SEQ ID NO: 53: CAATGGCTAA TACAAACTGC  
TGCAAAATCG,  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUUU CCUCAUUCG,  
SEQ ID NO: 62: CGAATGAGGAACTCAGTCTAAT-  
GAGAATT AGATTACTG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
AAUGAGAAUU AGAUUACUG,

65



67

SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUCC  
ACUCUGAUAU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTTC CCATCC, and  
SEQ ID NO: 111 GUGAUCAUUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC;

wherein said hybridization assay probe hybridizes to *Ureaplasma urealyticum* nucleic acid to form a detectable probe:target hybrid under stringent hybridization assay conditions, but does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

11. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

12. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

13. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

14. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45.

15. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, and SEQ ID NO: 53.

16. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 109, SEQ ID NO: 110, and SEQ ID NO: 111.

17. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63.

18. The hybridization assay probe of any one of claims 11, 12, 13, 14, 15, 16, and 17, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

19. A hybridization assay probe 10 to 100 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* biotype specific target nucleic acid sequence to form a detectable probe:target hybrid under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 nucleic acid or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not form said detectable probe:target hybrid with both *Ureaplasma urealyticum* biotype 1 nucleic acid and *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions, said biotype specific target nucleic acid sequence being selected from the group consisting of:

SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG,  
and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG; and  
wherein said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*,

68

*Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

20. The probe of claim 19, wherein said biotype specific target nucleic acid sequence is either SEQ ID NO: 126 or SEQ ID NO: 136.

21. The probe of claim 19, wherein said biotype specific target nucleic acid sequence is either SEQ ID NO: 127 or SEQ ID NO: 137.

22. A hybridization assay probe for distinguishing between different *Ureaplasma urealyticum* biotypes which is 20 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 131: GCTCGAACGAGTCGGTGTG, and  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG;

provided that under stringent hybridization assay conditions said hybridization assay probe hybridizes with *Ureaplasma urealyticum* biotype 2 nucleic acid to form a detectable probe:target hybrid, and said hybridization assay probe does not form said detectable probe:target hybrid with *Ureaplasma urealyticum* biotype 1 nucleic acid under said stringent hybridization assay conditions,

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

23. The probe of claim 22, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

24. A hybridization assay probe for distinguishing between different *Ureaplasma urealyticum* biotypes which is 18 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 122: CAACACCGAC CCATTCGG,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 132: CCGAATGGGT CGGTGTG, and  
SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG

provided that under stringent hybridization assay conditions said hybridization assay probe hybridizes with *Ureaplasma urealyticum* biotype 1 nucleic acid to form a detectable probe:target hybrid, and said hybridization assay probe does not form said detectable probe:target hybrid with *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions,

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

25. The probe of claim 24, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

26. A probe mix comprising:

a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 21 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,

69

SEQ ID NO: 8: CGTTAAGCAT CTAGATTAA TAC-  
CAAAC TTACAAACCCG,  
SEQ ID NO: 9: CCTACTACAC TCTAGGTTA  
CAGTTTTGA TACAGCTAGA,  
SEQ ID NO: 11: GTCAGTGATA GTCCAAGTTG  
GC,  
SEQ ID NO: 20: CGATTTTGCA GCAGTTTGTA  
TTAGCCATTG,  
SEQ ID NO: 26: GGATGGGAAC AGGTATTTCC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA  
GACTGAGTTT CCTCATTCG,  
SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC  
G,  
SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG  
U,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
UACCAAACUU ACAAAACCCG,  
SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUTUGGUUAU  
UAAAUCUAGA UGCUUAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAAC TG  
TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUUGUA UCAAAAACUG  
UAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGUAU GUCCAAGUUG  
GC,  
SEQ ID NO: 44: GCCAACTTGG ACTATCACTG  
AC,  
SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG  
AC,  
SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
UUAGCCAUG,  
SEQ ID NO: 53: CAATGGCTAA TACAAAAC TG  
TGCAAAAATCG,  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUU CCUCAUUCG,  
SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT  
AATGAGAATT AGATTACTG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
AAUGAGAAUU AGAUUACUG,  
SEQ ID NO: 109: GGAUGGGAAC AGGUUUUCC  
ACUCUGAUAU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGA  
ATACCTGTTC CCATCC, and  
SEQ ID NO: 111: GUGAUCAUUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and  
b) a helper probe.

27. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 1: TCATTGACTT GGTGAGCCAT  
TACCTCAC,

70

SEQ ID NO: 3: GCCGTGTCTC AGTCCCATTG  
TGGCTGTTCT,  
SEQ ID NO: 64: UCAUUGACUU GGUGAGCCAU  
UACCUCAC,  
SEQ ID NO: 65: GTGAGGTAAT GGCTACCAA  
GTCAATGA,  
SEQ ID NO: 66: GUGAGGUAAU GGCUCACCAA  
GUCAAUGA,  
SEQ ID NO: 67: GCCGUGUCUC AGUCCCAUUG  
UGGUGUUCU,  
SEQ ID NO: 68: AGAACAGCCA CAATGGGACT  
GAGACACGGC, and  
SEQ ID NO: 69: AGAACAGCCA CAAUGGGACU  
GAGACACGGC.

28. The probe mix of claim 27, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 2 or SEQ ID NO: 31;
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 1 or SEQ ID NO: 64; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 3 or SEQ ID NO: 67; and
- (b) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 32 or SEQ ID NO: 33;
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 65 or SEQ ID NO: 66; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 68 or SEQ ID NO: 69.

29. A probe mix comprising:

- a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is up to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36; wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

- b) a helper probe comprising a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 4: ATATAAAAGA ACTTTACAAT  
CTATAAGACC TTCATCGTTC ACGCGGC,  
SEQ ID NO: 70: AUUAUAAAAGA ACUUUACAAU  
CUAUAAGACC UUCAUCGUUUU ACGCGGC,  
SEQ ID NO: 71: GCCGCGTGAA CGATGAAGGT  
CTTATAGATT GTAAAGTTCT TTTATAT, and  
SEQ ID NO: 72: GCCGCGUGAA CGAUGAAGGU  
CUUAUAGAUU GUAAAGUUCU UUUUAU.

30. The probe mix of claim 29, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 5 or SEQ ID NO: 34;

71

- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 4 or SEQ ID NO: 70; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 6 or SEQ ID NO: 73; and

- (b) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 35 or SEQ ID NO: 36; 10
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 71 or SEQ ID NO: 72; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 74 or SEQ ID NO: 75. 15

31. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 7: CCTGCGCTCG TTTTACGCC  
AGTAAATCCG GATAACGC,  
SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA 25  
CAGTTTTTGA TACAGCTAGA,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAACCTG  
TAAACCTAGA GTGTAGTAGG, 30  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
UAAACCUGA GUGUAGUAGG,  
SEQ ID NO: 76: CCUGCGCUCG UUUUACGCC  
AGUAAAUCCG GAUAACGC, 35  
SEQ ID NO: 77: GCGTTATCCG GATTTACTGG GCG-  
TAAAACG AGCGCAGG, and  
SEQ ID NO: 78: GCGUUAUCCG GAUUUACUGG  
GCGUAAAACG AGCGCAGG.

32. The probe mix of claim 31, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 8 or SEQ ID NO: 37; 45
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 7 or SEQ ID NO: 76; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 9 or SEQ ID NO: 40; 50
- (b) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 38 or SEQ ID NO: 39; 55
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 77 or SEQ ID NO: 78; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 41 or SEQ ID NO: 42. 60

33. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

72

SEQ ID NO: 8: CGTTAAGCAT CTAGATTTAA TAC-  
CAAACCTT ACAAACCCG,  
SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT  
TCCATATATC TA,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 38: CGGGTTTGTGA AGTTTGGTAT  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUAU  
UAAAUCUAGA UGCUUAACG,  
SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU  
UCCAUUAUUC UA,  
SEQ ID NO: 80: TAGATATATG GAAGAACACC  
GGTGGCGAAG GC, and  
SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC  
GGUGGCGAAG GC.

34. The probe mix of claim 33, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 9 or SEQ ID NO: 40;
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 8 or SEQ ID NO: 37; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 10 or SEQ ID NO: 79; and
- (b) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 41 or SEQ ID NO: 42;
  - a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 38 or SEQ ID NO: 39; and
  - a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 80 or SEQ ID NO: 81.

35. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT  
TCCATATATC TA,  
SEQ ID NO: 12: CTAATCCTAT TTGCTCCCCA  
CACTTCGAG CCTAAGC,  
SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU  
UCCAUUAUUC UA,  
SEQ ID NO: 80: TAGATATATG GAAGAACACC  
GGTGGCGAAG GC,  
SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC  
GGUGGCGAAG GC,  
SEQ ID NO: 82: CUAUCCUAU UUGCUCUCCCA  
CACUUUCGAG CCUAAGC,  
SEQ ID NO: 83: GCTTAGGCTC GAAAGTGTGG  
GGAGCAAATA GGATTAG, and  
SEQ ID NO: 84: GCUUAGGCUC GAAAGUGUGG  
GGAGCAAUA GGAUUAG.

36. The probe mix of claim 35, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising
  - a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 11 or SEQ ID NO: 43;

73

- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 10 or SEQ ID NO: 79; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 12 or SEQ ID NO: 82; and
- (b) a probe mix comprising
- a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 44 or SEQ ID NO: 45;
- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 80 or SEQ ID NO: 81; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 83 or SEQ ID NO: 84.
37. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:
- SEQ ID NO: 19: TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG,
- SEQ ID NO: 21: CGAATTGCAG CCCTCTATCC GAAGTGAAGC TAACCTTTTC TG,
- SEQ ID NO: 97: UAGCACGUUU GCAGCCCUAG AUUAAGGGG CAUGAUG,
- SEQ ID NO: 98: CATCATGCCC CTTATATCTA GGGCTGCAAA CGTGCTA,
- SEQ ID NO: 99: CAUCAUGCCC CUUAUAUCUA GGCUGCAAA CGUGCUA,
- SEQ ID NO: 100: CGAAUUGCAG CCCUCUAUCC GAACUGAGAC UAACUUUUUC UG,
- SEQ ID NO: 101: CAGAAAAAGT TAGTCTCAGT TCGGATAGAG GGCTGCAATT CG, and,
- SEQ ID NO: 102: CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAUU CG.
38. The probe mix of claim wherein 37, said probe mix is selected from the group consisting of:
- (a) a probe mix comprising
- a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 20 or SEQ ID NO: 52;
- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 19 or SEQ ID NO: 97; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 21 or SEQ ID NO: 100; and
- (b) a probe mix comprising
- a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 53 or SEQ ID NO: 54;
- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 98 or SEQ ID NO: 99; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 101 or SEQ ID NO: 102.
39. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

74

- SEQ ID NO: 28: GAGATCAACG GATTAAAGCC TCT-TATCAGC TACCCGTTGC TTATCGCAGA TTAGCACG,
- SEQ ID NO: 30: CACTTCACCA GGTATCGCTC TGT-TAAACTA TGAATTCATT TATA,
- SEQ ID NO: 115: GAGAUCAACG GAUUAAGGCC UCUUAUCAGC UACCCGUUGC UUAUCGCAGA UUAGCACG,
- SEQ ID NO: 116: CGTGCTAATC TGCATAAGC AACGGGTAGC TGATAAGAGG CTTTAATCCG TTGATCTC,
- SEQ ID NO: 117: CGUGCUAAUC UGCGAUAAGC AACGGGUAGC UGAUAAGAGG CUUUAUCCG UUGAUCUC,
- SEQ ID NO: 118: CACUUCACCA GGUAUCGCUC UGUUAAACUA UGAAUUAUU UAUA,
- SEQ ID NO: 119: TATAAATGAA TTCATAGTTT AACAGAGCGA TACCTGGTGA AGTG, and
- SEQ ID NO: 120: UAUAAAUGAA UUCAUAGUUU AACAGAGCGA UACCUUGUGA AGUG.
40. The probe mix of claim 39, wherein said probe mix is selected from the group consisting of:
- (a) a probe mix comprising
- a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 29 or SEQ ID NO: 61;
- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 28 or SEQ ID NO: 115; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 30 or SEQ ID NO: 118; and
- (a) a probe mix comprising
- a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 62 or SEQ ID NO: 63;
- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 116 or SEQ ID NO: 117; and
- a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 119 or SEQ ID NO: 120.
41. A probe mix comprising
- a hybridization assay probe 18 to 100 nucleotides in length comprising a nucleotide base sequence selected from the group consisting of:
- SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,
- SEQ ID NO: 122: CAACACCGAC CCATTCGG,
- SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,
- SEQ ID NO: 127: CAACACCGAC CCAUUCGG,
- SEQ ID NO: 131: GCTCGAACGA GTCGGTGTG,
- SEQ ID NO: 132: CCGAATGGGT CGGTGTG,
- SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG, and
- SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;
- provided that said hybridization assay probe forms a detectable probe:target hybrid under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 nucleic acid or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not form said detectable probe:target hybrid with both *Ureaplasma urealyticum* biotype 1 nucleic acid and *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions,

75

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions; and

a helper probe comprising a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 123: CGACATTTAA TGATGATCGT  
TTACGGTGTG GAC,

SEQ ID NO: 124: GCCGACATTT AATGATGATC  
GTTTACGGTG TGGAC,

SEQ ID NO: 125: CCCAGGCACA TCATTTAATG  
CGTAGCTA,

SEQ ID NO: 128: CGACAUUUA UGAUGAUCGU  
UUACGGUGUG GAC,

SEQ ID NO: 129: GCCGACAUUU AAUGAUGAUC  
GUUUACGGUG UGGAC,

SEQ ID NO: 130: CCCAGGCACA UCAUUUAAUG  
CGUAGCUA,

SEQ ID NO: 133: GTCCACACCG TAAACGATCA  
TCATTAAATG TCG,

SEQ ID NO: 134: GTCCACACCG TAAACGATCA  
TCATTAAATG TCGGC,

SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT  
GTGCCTGGG,

SEQ ID NO: 138: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCG,

SEQ ID NO: 139: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCGGC, and

SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU  
GUGCCUGGG.

42. The probe mix of claim 41, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 121 or SEQ ID NO: 126,

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 123 or SEQ ID NO: 128; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 125 or SEQ ID NO: 130; and

(b) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 131 or SEQ ID NO: 136;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 133 or SEQ ID NO: 138; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 135 or SEQ ID NO: 140.

43. A probe mix selected from the group consisting of:

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 122 or SEQ ID NO: 127,

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 124 or SEQ ID NO: 129; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 125 or SEQ ID NO: 130; and

76

(b) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 132 or SEQ ID NO: 137;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 134 or SEQ ID NO: 139; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 135 or SEQ ID NO: 140.

44. A probe mix comprising

a) a hybridization assay probe for detecting *Ureaplasma urealyticum* under stringent hybridization assay conditions which is 28 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 14: CGTTCGAGCC GACATTTAAT  
GATGATCG,

SEQ ID NO: 46: CGUUCGAGCC GACAUUUAAU  
GAUGAUCG,

SEQ ID NO: 47: CGATCATCAT TAAATGTCCG  
CTCGAACG, and

SEQ ID NO: 48: CGAUCAUCAU UAAAUGUCGG  
CUCGAACG;

wherein under said stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

b) a helper probe consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 13: TTTACGGTGT GGACTACTAG  
GGTAT,

SEQ ID NO: 15: GCGTTAGCTA CAACACCGAC T,  
SEQ ID NO: 85: UUUACGGUGU GGACUACUAG  
GGUAU,

SEQ ID NO: 86: ATACCCTAGT AGTCCACACC  
GTAAA,

SEQ ID NO: 87: AUACCCUAGU AGUCCACACC  
GUAAA,

SEQ ID NO: 88: GCGUUAGCUA CAACACCGAC  
U,

SEQ ID NO: 89: AGTCGGTGTT GTAGCTAACG C,  
and

SEQ ID NO: 90: AGUCGGUGUU GUAGCUAACG  
C.

45. The probe mix of claim 44, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 14 or SEQ ID NO: 46;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 13 or SEQ ID NO: 85; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 15 or SEQ ID NO: 88; and

(b) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 47 or SEQ ID NO: 48;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 86 or SEQ ID NO: 87; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 89 or SEQ ID NO: 90.

46. A probe mix comprising

- a) a hybridization assay probe for detecting *Ureaplasma urealyticum* under stringent hybridization assay conditions which is 24 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:  
 SEQ ID NO: 17: GCGTCGCAAT AGATGTCAAA  
 CCTAG,  
 SEQ ID NO: 49: GCGUCGCAAU AGAUGUCAA  
 CCUAG,  
 SEQ ID NO: 50: CTAGGTTTGA CATCTATTGC  
 GACGC, and  
 SEQ ID NO: 51: CUAGGUUUGA CAUCUAUUGC  
 GACGC;

wherein under said stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with a *Ureaplasma urealyticum* target nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

- b) a helper probe consisting of a nucleotide base sequence selected from the group consisting of:  
 SEQ ID NO: 16: GTAAGGTCTT ACGTGATTG  
 TCAAATTAAG CAACATGCTC CACCAC,  
 SEQ ID NO: 18: CGACAACCAT GCACCACCTG  
 TCATATTGTT AACCTCAAC,  
 SEQ ID NO: 91: GUAAGGUUCU ACGUGUAUUG  
 UCAAAUUAAG CAACAUGCUC CACCAC,  
 SEQ ID NO: 92: GTGGTGGAGC ATGTTGCTTA  
 ATTTGACAAT ACACGTAGAA CCTTAC,  
 SEQ ID NO: 93: GUGGUGGAGC AUGUUGCUUA  
 AUUUGACAAU ACACGUAGAA CCUAC,  
 SEQ ID NO: 94: CGACAACCAU GCACCACCUG  
 UCAUAUUGUU AACCUAAC,  
 SEQ ID NO: 95: GTTGAGGTTA ACAATATGAC  
 AGGTGGTGCA TGGTTGTCG, and  
 SEQ ID NO: 96: GUUGAGGUUA ACAAUAGAC  
 AGGUGGUGCA UGGUUGUCG.

47. The probe mix of claim 46, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising  
 a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 17 or SEQ ID NO: 49;  
 a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 16 or SEQ ID NO: 91; and  
 a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 18 or SEQ ID NO: 94; and  
 (b) a probe mix comprising  
 a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 50 or SEQ ID NO: 51;  
 a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 92 or SEQ ID NO: 93; and  
 a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 95 or SEQ ID NO: 96.

48. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Myco-*

*plasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis* comprising the steps of:

- a) providing to said sample a hybridization assay probe comprising an oligonucleotide which under stringent hybridization assay conditions hybridizes to a *Ureaplasma urealyticum* target nucleic acid selected from the group consisting of  
 SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC  
 G,  
 SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG  
 U,  
 SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
 UACCAAACUU ACAAACCCG,  
 SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUA  
 UAAAUUCUAGA UGCUUAACG,  
 SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
 CAGUUUUUGA UACAGCUAGA,  
 SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
 UAAACCUAGA GUGUAGUAGG,  
 SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG  
 GC,  
 SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG  
 AC,  
 SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
 UUAGCCAUUG,  
 SEQ ID NO: 54: CAAUGGCUAA UACAAACUGC  
 UGCAAAAUCG,  
 SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU  
 GCUUGACUUC UGUGUUCGGG AUG,  
 SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA  
 AGCACUCUAG AGCCGAAAAU AGC,  
 SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU  
 UCUGUGUUCG,  
 SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC  
 UCUAGAGCCG,  
 SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
 GACUGAGUUU CCUCAUUCG,  
 SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
 AAUGAGAAU AGAUUACUG,  
 SEQ ID NO: 109: GGAUGGGAAC AGGUUUUCC  
 ACUCUGAUUA GAUCAC, and  
 SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA  
 AUACCUGUUC CCAUCC;

wherein under said stringent hybridization assay conditions said oligonucleotide hybridizes with said target nucleic acid to form a detectable probe:target hybrid and does not hybridize to form a detectable probe:non-target hybrid with *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* nucleic acid under said stringent hybridization assay conditions; and

- b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

49. The method of claim 48, wherein target nucleic acid is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.

50. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.

51. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.

52. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.

79

53. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 52 and SEQ ID NO: 54.

54. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 57.

55. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 58 and SEQ ID NO: 60.

56. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.

57. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.

58. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

- a) providing to said sample a hybridization assay probe comprising a detection nucleotide base sequence selected from the group consisting of:
  - SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,
  - SEQ ID NO: 8: CGTTAAGCAT CTAGATTAA TAC-CAAACCTT ACAAACCCG,
  - SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA CAGTTTGTGA TACAGCTAGA,
  - SEQ ID NO: 11: GTCAGTGATA GTCCAAGTTG GC,
  - SEQ ID NO: 20: CGATTTTGCA GCAGTTTGTA TTAGCCATTG,
  - SEQ ID NO: 22: GCTATTTTCG GCTCTAGAGT GCTTGACTTC TGTGTTCCGGG ATG,
  - SEQ ID NO: 23: CGGCTCTAGA GTGCTTGACT TCTGTGTTCCG,
  - SEQ ID NO: 26: GGATGGGAAC AGGTATTTC ACTCTGATAT GATCAC,
  - SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA GACTGAGTTT CCTCATTCG,
  - SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,
  - SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,
  - SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,
  - SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA UACCAAACUU ACAAACCCG,
  - SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT TAAATCTAGA TGCTTAACG,
  - SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUU UAAAUCUAGA UGCUUAAACG,
  - SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA,
  - SEQ ID NO: 41: TCTAGCTGTA TCAAAAACCTG TAAACCTAGA GTGTAGTAGG,
  - SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,
  - SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG GC,
  - SEQ ID NO: 44: GCCAACTTGG ACTATCACTG AC,
  - SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,
  - SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA UUAGCCAUUG,
  - SEQ ID NO: 53: CAATGGCTAA TACAACTGC TGCAAAATCG,
  - SEQ ID NO: 54: CAAUGGCUAA UACAAACUGC UGCAAAAUCG,

80

SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU GCUUGACUUC UGUGUUCGGG AUG,  
 SEQ ID NO: 56: CATCCGAAC ACAGAAGTCA AGCACTCTAG AGCCGAAAAT AGC,  
 SEQ ID NO: 57: CAUCCGAAC ACAGAAGUCA AGCACUCUAG AGCCGAAAAU AGC,  
 SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU UCUGUGUUCG,  
 SEQ ID NO: 59: CGAACACAGA AGTCAAGCAC TCTAGAGCCG,  
 SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC UCUAGAGCCG,  
 SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA GACUGAGUUU CCUCAUUCG,  
 SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT AATGAGAATT AGATTACTG,  
 SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU AAUGAGAAU AGAUUACUG,  
 SEQ ID NO: 109: GGAUGGGAAC AGGUUUUCC ACUCUGAUU AU GAUCAC,  
 SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA ATACCTGTTC CCATCC, and  
 SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions said hybridization assay probe hybridizes with nucleic acid from *Ureaplasma urealyticum* to form a probe:target hybrid and does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

59. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

60. The method of claim 59, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 1: TCATTGACTT GGTGAGCCAT TACCTCAC,  
 SEQ ID NO: 3: GCCGTGTCTC AGTCCCAITG TGGCTGTTCT,  
 SEQ ID NO: 64: UCAUUGACUU GGUGAGCCAU UACCUCAC,  
 SEQ ID NO: 65: GTGAGGTAAT GGCTCACCAA GTCAATGA,  
 SEQ ID NO: 66: GUGAGGUAAU GGCUCACCAA GUCAAUGA,  
 SEQ ID NO: 67: GCCGUGUCUC AGUCCCAUUG UGGCUGUUCU,  
 SEQ ID NO: 68: AGAACAGCCA CAATGGGACT GAGACACGGC, and  
 SEQ ID NO: 69: AGAACAGCCA CAAUGGGACU GAGACACGGC.

61. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

- a) providing to said sample a hybridization assay probe comprising a detection nucleotide base sequence

81

selected from the group consisting of: SEQ ID NO: 5, SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36; wherein under stringent hybridization assay conditions said hybridization assay probe hybridizes with nucleic acid from *Ureaplasma urealyticum* to form a probe:target hybrid and does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample;

wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 4: ATATAAAGA ACTTTACAAT CTATAAGACC TTCATCGTTC ACGCGGC,  
SEQ ID NO: 6: GGCACATAGT TAGCCGATAC TTATCAAAT GGTACAGTCA AA,  
SEQ ID NO: 70: AUAUAAAAGA ACUUUACAAU CUUAAGACC UCAUCGUUC ACGCGGC,  
SEQ ID NO: 71: GCCGCGTGAA CGATGAAGGT CTTATAGATT GTAAAGTTCT TTTATAT,  
SEQ ID NO: 72: GCCGCGUGAA CGAUGAAGGU CUUAUAGAUU GUAAAGUUCU UUUUAUUAU,  
SEQ ID NO: 73: GGCACAUAGU UAGCCGAUAC UUAUUAUUAU GGUACAGUCA AA,  
SEQ ID NO: 74: TTTGACTGTA CCATTTGAAT AAGTATCGGC TAACTATGTG CC, and  
SEQ ID NO: 75: UUUGACUGUA CCAUUUGAAU AAGUAUCGGC UAACUAUGUG CC.

62. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

63. The method of claim 62, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 7: CCTGCGCTCG TTTTACGCCC AGTAAATCCG GATAACGC,  
SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA CAGTTTTTGA TACAGCTAGA,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAACCTG TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 76: CCUGCGCUCG UUUUACGCCC AGUAAAUCCG GAUAACGC,  
SEQ ID NO: 77: GCGTTATCCG GATTACTGG GCG-TAAAACG AGCGCAGG, and  
SEQ ID NO: 78: GCGUUAUCCG GAUUUACUGG GCGUAAAACG AGCGCAGG.

64. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

65. The method of claim 64, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 8: CGTTAAGCAT CTAGATTTAA TAC-CAAACCTT ACAAACCCG,

82

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT TCCATATATC TA,

SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA UACCAAACUU ACAAACCCG,

SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT TAAATCTAGA TGCTTAACG,

SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUA UAAAUUAGA UGCUUAACG,

SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU UCCAUAUAUC UA,

SEQ ID NO: 80: TAGATATATG GAAGAACACC GGTGGCGAAG GC, and

SEQ ID NO: 81: UAGAUUAUUG GAAGAACACC GGUGGCGAAG GC.

66. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45.

67. The method of claim 66, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT TCCATATATC TA,

SEQ ID NO: 12: CTAATCCTAT TTGCTCCCCA CACTTTCGAG CCTAAGC,

SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU UCCAUAUAUC UA,

SEQ ID NO: 80: TAGATATATG GAAGAACACC GGTGGCGAAG GC,

SEQ ID NO: 81: UAGAUUAUUG GAAGAACACC GGUGGCGAAG GC,

SEQ ID NO: 82: CUAAUCCUUA UUGCUCUCCCA CACUUUCGAG CCUAAGC,

SEQ ID NO: 83: GCTTAGGCTC GAAAGTGTGG GGAGCAAATA GGATTAG, and

SEQ ID NO: 84: GCUUAGGCUC GAAAGUGUGG GGAGCAAUA GGAUUAAG.

68. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54.

69. The method of claim 68, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 19: TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG,

SEQ ID NO: 21: CGAATTGCAG CCCTCTATCC GAACTGAGAC TAACTTTTTC TG,

SEQ ID NO: 97: UAGCACGUUU GCAGCCCUAG AUAUAAGGGG CAUGAUG,

SEQ ID NO: 98: CATCATGCCC CTTATATCTA GGGCTGCAAA CGTGCTA,

SEQ ID NO: 99: CAUCAUGCCC CUUAUAUCUA GGGCUGCAAA CGUGCUA,

SEQ ID NO: 100: CGAAUUGCAG CCCUCUAUCC GAACUGAGAC UAACUUUUUC UG,

SEQ ID NO: 101: CAGAAAAAGT TAGTCTCAGT TCGGATAGAG GGCTGCAATT CG, and,

SEQ ID NO: 102: CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAUU CG.

70. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 22, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57.



71. The method of claim 70, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 24: GGAACAGGTA TTTCCACTCT  
GATATGATCA CTAC,

SEQ ID NO: 25: GCGTAGCGAT GACCTATTTT  
ACTTGC,

SEQ ID NO: 103: GGAACAGGUA UUUCCACUCU  
GAUAUGAUA CUAC,

SEQ ID NO: 104: GTAGTGATCA TATCAGAGTG  
GAAATACCTG TTCC,

SEQ ID NO: 105: GUAGUGAUA UAUCAGAGUG  
GAAAUACCUG UUC,

SEQ ID NO: 106: GCGUAGCGAU GACCUAUUUU  
ACUUGC,

SEQ ID NO: 107: GCAAGTAAAA TAGGTCATCG  
CTACGC, and

SEQ ID NO: 108: GCAAGUAAAA UAGGUCAUCG  
CUACGC.

72. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60.

73. The method of claim 72, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 26: GGATGGGAAC AGGTATTTCC  
ACTCTGATAT GATCAC,

SEQ ID NO: 27: GCGTAGCGAT GACCTATTTT ACT-  
TGCGCTA TTTT,

SEQ ID NO: 109: GGAUGGGAAC AGGUUUC  
ACUCUGAUU GAUCAC,

SEQ ID NO: 110: GTGATCATAT CAGAGTGGA  
ATACCTGTTC CCATCC,

SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA  
AUACCUGUUC CCAUCC,

SEQ ID NO: 112: GCGUAGCGAU GACCUAUUUU  
ACUUGCGCUA UUUU,

SEQ ID NO: 113: AAAATAGCGC AAGTAAATA  
GGTCATCGCT ACGC, and

SEQ ID NO: 114: AAAUAGCGC AAGUAAAA  
GGUACUCCU ACGC.

74. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63.

75. The method of claim 74, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 28: GAGATCAACG GATTAAAGCC TCT-  
TATCAGC TACCCGTTGC TTATCGCAGA  
TTAGCACG,

SEQ ID NO: 30: CACTTCACCA GGTATCGCTC TGT-  
TAAACTA TGAATTCATT TATA,

SEQ ID NO: 115: GAGAUCAACG GAUUAAGCC  
UCUUAUCAGC UACCCGUUGC UUAUCGCAGA  
UUAGCACG,

SEQ ID NO: 116: CGTGCTAATC TCGATAAGC  
AACGGGTAGC TGATAAGAGG CTTTAATCCG  
TTGATCTC,

SEQ ID NO: 117: CGUGCUAAUC UGCGAUAAAGC  
AACGGGUAGC UGAUAAGAGG CUUUAUCCG  
UUGAUCUC,

SEQ ID NO: 118: CACUUCACCA GGUAUCGCUC  
UGUAAAACUA UGAAUUCAUU UAUA,

SEQ ID NO: 119: TATAAATGAA TTCATAGTTT  
AACAGAGCGA TACCTGGTGA AGTG, and

SEQ ID NO: 120: UAUAUAUGAA UUCAUAGUUU  
AACAGAGCGA UACCUGGUGA AGUG.

76. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 109, SEQ ID NO: 110, and SEQ ID NO: 111.

77. The method of any one of claims 59, 60, and 61-76, wherein said hybridization assay probe consists of said detection nucleotide base sequence and one or more reporter groups.

78. A method for specifically detecting the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 comprising the steps of:

a) contacting a sample with a hybridization assay probe able to hybridize under stringent hybridization assay conditions to a *Ureaplasma urealyticum* biotype specific target nucleic acid sequence to form a probe:target hybrid with either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not hybridize to nucleic acid from both *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2 under said stringent hybridization assay conditions to form a detectable probe:non-target hybrid, said *Ureaplasma urealyticum* biotype specific target nucleic acid sequence being selected from the group consisting of:

SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,

SEQ ID NO: 127: CAACACCGAC CCAUUCGG,

SEQ ID NO: 136: GCUCGAACGA GUCCGUGUUG,

and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;

provided that under said stringent hybridization conditions said probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication of the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2.

79. The method of claim 78, wherein said target nucleic acid sequence is either SEQ ID NO: 126 or SEQ ID NO: 136.

80. The method of claim 78, wherein said target nucleic acid sequence is either SEQ ID NO: 127 or SEQ ID NO: 137.

81. A method for specifically detecting the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 comprising the steps of:

a) contacting a sample with a hybridization assay probe able to hybridize under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 nucleic acid to form a detectable probe:target hybrid, wherein said hybridization assay probe does not hybridize to nucleic acid from both *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2 under said stringent hybridization assay conditions to form said detectable probe:non-target hybrid, said hybridization assay probe

comprising a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
SEQ ID NO: 122: CAACACCGAC CCATTCGG,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 131: GCTCGAACGA GTCGGTGTG,  
SEQ ID NO: 132: CCGAATGGGT CGGTGTG,  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG,  
and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;

provided that under said stringent hybridization conditions said probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication of the presence of either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2.

82. The method of claim 81, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 131: GCTCGAACGA GTCGGTGTG, and  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG.

83. The method of claim 82, further comprising the use of a helper probe consisting of a sequence selected from the group consisting of:

SEQ ID NO: 123: CGACATTTAA TGATGATCGT  
TTACGGTGTG GAC,  
SEQ ID NO: 125: CCCAGGCACA TCATTTAATG  
CGTTAGCTA,  
SEQ ID NO: 128: CGACAUUUA UGAUGAUCGU  
UUACGGUGUG GAC,  
SEQ ID NO: 130: CCCAGGCACA UCAUUUAAUG  
CGUUAGCUA,  
SEQ ID NO: 133: GTCCACACCG TAAACGATCA  
TCATTAAATG TCG,  
SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT  
GTGCCTGGG,  
SEQ ID NO: 138: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCG, and  
SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU  
GUGCCUGGG.

84. The method of claim 81, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 122: CAACACCGAC CCATTCGG,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 132: CCGAATGGGT CGGTGTG, and  
SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG.

85. The method of claim 84, further comprising the use of a helper probe in said step (a), said helper probe consisting of a sequence selected from the group consisting of:

SEQ ID NO: 124: GCCGACATTT AATGATGATC GTT-  
TACGGTG TGGAC,  
SEQ ID NO: 125: CCCAGGCACA TCATTTAATG  
CGTTAGCTA,  
SEQ ID NO: 129: GCCGACAUUU AAUGAUGAUC  
GUUUACGGUG UGGAC,

SEQ ID NO: 130: CCCAGGCACA UCAUUUAAUG  
CGUUAGCUA,

SEQ ID NO: 134: GTCCACACCG TAAACGATCA  
TCATTAAATG TCGGC,

SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT  
GTGCCTGGG,

SEQ ID NO: 139: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCGGC, and

SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU  
GUGCCUGGG.

86. The method of any one of claims 82-85, wherein said hybridization assay probe consists of one or more reporter groups and said detection nucleotide base sequence.

87. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 14: CGTTCGAGCC GACATTTAAT  
GATGATCG,

SEQ ID NO: 46: CGUUCGAGCC GACAUUUAU  
GAUGAUCG,

SEQ ID NO: 47: CGATCATCAT TAAATGTCGG  
CTCGAACG, and

SEQ ID NO: 48: CGAUCAUCAU UAAAUGUCGG  
CUCGAACG; wherein under stringent hybridiza-

tion assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but not with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae*; and

a helper probe consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 13: TTTACGGTGT GGACTACTAG  
GGTAT,

SEQ ID NO: 15: GCGTTAGCTA CAACACCGAC T,  
SEQ ID NO: 85: UUUACGGUGU GGACUACUAG  
GGUAU,

SEQ ID NO: 86: ATACCCTAGT AGTCCACACC  
GTAAA,

SEQ ID NO: 87: AUACCCUAGU AGUCCACACC  
GUAAA,

SEQ ID NO: 88: GCGUUAGCUA CAACACCGAC  
U,

SEQ ID NO: 89: AGTCGGTGTT GTAGCTAACG C,  
and

SEQ ID NO: 90: AGUCGGUGUU GUAGCUAACG  
C; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

88. The method of claim 87, wherein said hybridization assay probe consists of one or more reporter groups and a nucleotide base sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48.

89. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis* comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 17: GCGTCGCAAT AGATGTCAAA CCTAG,  
 SEQ ID NO: 49: GCGUCGCAAU AGAUGUCAAA CCUAG,  
 SEQ ID NO: 50: CTAGGTTTGA CATCTATTGC GACGC, and  
 SEQ ID NO: 51: CUAGGUUUGA CAUCUAUUGC GACGC; wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but not with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae*; and a helper probe consisting of a nucleotide base sequence selected from the group consisting of:  
 SEQ ID NO: 16: GTAAGGTTCT ACGTGTTATG TCAAATTAAG CAACATGCTC CACCAC,  
 SEQ ID NO: 18: CGACAACCAT GCACCACCTG TCATATTGTT AACCTCAAC,  
 SEQ ID NO: 91: GUAAGGUUCU ACGUGUAUUG UCAAAUUAAG CAACAUGCUC CACCAC,  
 SEQ ID NO: 92: GTGGTGGAGC ATGTTGCTTA ATTTGACAAT ACACGTAGAA CCTTAC,  
 SEQ ID NO: 93: GUGGUGGAGC AUGUUGCUUA AUUUGACAAU ACACGUAGAA CCUAC,  
 SEQ ID NO: 94: CGACAACCAU GCACCACCG UCAUAUUGUU AACCUCAAC,  
 SEQ ID NO: 95: GTTGAGGTTA ACAATATGAC AGGTGGTGCA TGGTTGTCG, and  
 SEQ ID NO: 96: GUUGAGGUUA ACAUAUUGAC AGGUGGUGCA UGGUUGUCG; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

90. The method of claim 89, wherein said hybridization assay probe consists of one or more reporter groups and a nucleotide base sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51.

91. A hybridization assay probe 10 to 50 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* target nucleic acid sequence to form a detectable probe:target hybrid with said *Ureaplasma urealyticum* target nucleic acid sequence under stringent hybridization assay conditions, wherein said *Ureaplasma urealyticum* target nucleic acid sequence is selected from the group consisting of:

SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,  
 SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
 SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA UACCAAACUU ACAAACCCG,  
 SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUAU UAAAUCUAGA UGCUUAACG,  
 SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA,  
 SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,  
 SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG GC,  
 SEQ ID NO: 45: GCCAACUUUG ACUAUCACUG AC,  
 SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU GCUUUGACUUC UGUGUUCGGG AUG,  
 SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA AGCACUCUAG AGCCGAAAAU AGC,

SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU UCUGUGUUCG,  
 SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC UCUAGAGCCG,  
 SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA GACUGAGUUU CCUCAUUUCG,  
 SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU AAUGAGAAUU AGAUTUACUG,  
 SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUCC ACUCUGAUAU GAUCAC, and  
 SEQ ID NO: 111: GUGAUCUAU CAGAGUGGAA AUACCUGUCC CCAUCC;

wherein under said stringent hybridization assay conditions said hybridization assay probe does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma hominis*.

92. The hybridization assay probe of claim 91, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium* and *Mycoplasma pneumoniae*.

93. The hybridization assay probe of claim 91, wherein said hybridization assay probe also does not form said detectable probe:non target hybrid with nucleic acid from *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*.

94. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.

95. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.

96. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.

97. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.

98. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 57.

99. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 58 and SEQ ID NO: 60.

100. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.

101. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.

102. A probe mix comprising:

a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 10 to 50 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,  
 SEQ ID NO: 8: CGTTAAGCAT CTAGATTAA TAC-CAAACCT ACAAACCCG,

SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA  
CAGTTTTTGA TACAGCTAGA,  
SEQ ID NO: 11: GTCAGTGATA GTCCAAGTTG  
GC,  
SEQ ID NO: 20: CGATTTTGCA GCAGTTTGTA 5  
TTAGCCATTG,  
SEQ ID NO: 22: GCTATTTTCG GCTCTAGAGT  
GCTTGACTTC TGTGTTCCGGG ATG,  
SEQ ID NO: 23: CGGCTCTAGA GTGCTTGACT  
TCTGTGTTCC,  
SEQ ID NO: 26: GGATGGGAAC AGGTATTTC 10  
ACTCTGATAT GATCAC,  
SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA  
GACTGAGTTT CCTCATTCG,  
SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC 15  
G,  
SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG  
U,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUA 20  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGUAU  
UAAAUUCUAGA UGCUUAACG, 25  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAACTG  
TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG 30  
UAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG  
GC,  
SEQ ID NO: 44: GCCAACTTGG ACTATCACTG 35  
AC,  
SEQ ID NO: 45: GCCAACUUUGG ACUAUCACUG  
AC,  
SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
UUAGCCAUUG,  
SEQ ID NO: 53: CAATGGCTAA TACAACTGC 40  
TGCAAAATCG,  
SEQ ID NO: 54: CAAUGGCUAA UACAAACUGC  
UGCAAAAUCG,  
SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU  
GCUUGACUUC UGUGUUUCGGG AUG, 45  
SEQ ID NO: 56: CATCCCGAAC ACAGAAGTCA  
AGCACTTAG AGCCGAAAAT AGC,  
SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA  
AGCACUCUAG AGCCGAAAAU AGC,  
SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU 50  
UCUGUGUUCG,  
SEQ ID NO: 59: CGAACACAGA AGTCAAGCAC  
TCTAGAGCCG,  
SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC  
UCUAGAGCCG, 55  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUUU CCUCAUUUCG,  
SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT  
AATGAGAATT AGATTACTG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU 60  
AAUGAGAAUU AGAUUACUG,  
SEQ ID NO: 109: GGAUUGGGAAC AGGUAU-  
UCC ACUCUGAUAU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTTC CCATCC, and  
SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA 65  
AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and  
b) a helper probe.

103. The probe mix of claim 102, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 22, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57, and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 24: GGAACAGGTA TTTCCACTCT  
GATATGATCA CTAC,

SEQ ID NO: 25: GCGTAGCGAT GACCTATTTT  
ACTTGC,

SEQ ID NO: 103: GGAACAGGUA UUUCCACUCU  
GAUAUGAUA CUAC,

SEQ ID NO: 104: GTAGTGATCA TATCAGAGTG  
GAAATACCTG TTCC,

SEQ ID NO: 105: GUAGUGAUA UAUCAGAGUG  
GAAAUACCUG UUCC,

SEQ ID NO: 106: GCGUAGCGAU GACCUAUUUU  
ACUUGC,

SEQ ID NO: 107: GCAAGTAAAA TAGGTCATCG  
CTACGC, and

SEQ ID NO: 108: GCAAGUAAAA UAGGUCAUCG  
CUACGC.

104. The probe mix of claim 103, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising:

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 22 or SEQ ID NO: 55;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 24 or SEQ ID NO: 103; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 25 or SEQ ID NO: 106; and

(b) a probe mix comprising:

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 56 or SEQ ID NO: 57;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 104 or SEQ ID NO: 105; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 107 or SEQ ID NO: 108.

105. The probe mix of claim 102, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60, and said helper probe comprises a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 26: GGATGGGAAC AGGTATTTC  
ACTCTGATAT GATCAC,

SEQ ID NO: 27: GCGTAGCGAT GACCTATTTT ACT-  
TGCGCTA TTTT,

SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUCC  
ACUCUGAUAU GAUCAC,

SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTTC CCATCC,

91

SEQ ID NO: 111: GUGAUCUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC,

SEQ ID NO: 112: GCGUAGCGAU GACCUAULUU  
ACUUGCGCUA UUU,

SEQ ID NO: 113: AAAATAGCGC AAGTAAAATA 5  
GGTCATCGCT ACGC, and

SEQ ID NO: 114: AAAAUAGCGC AAGUAAAAUA,  
GGUCAUCGCU ACGC.

106. The probe mix of claim 105, wherein said probe mix  
is selected from the group consisting of:

(a) a probe mix comprising:

a hybridization assay probe consisting of one or more  
reporter groups and the nucleotide base sequence of  
either SEQ ID NO: 23 or SEQ ID NO: 58;

a first helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 26 or SEQ ID NO:  
109; and

a second helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 27 or SEQ ID NO: 20  
112; and

(a) a probe mix comprising:

a hybridization assay probe consisting of one or more  
reporter groups and the nucleotide base sequence of  
either SEQ ID NO: 59 or SEQ ID NO: 60;

92

a first helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 110 or SEQ ID NO:  
111; and

a second helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 113 or SEQ ID NO:  
114.

107. A hybridization assay probe 10 to 100 nucleotides in  
length comprising an oligonucleotide sufficiently comple-  
mentary to a *Ureaplasma urealyticum* target nucleic acid  
sequence to form a detectable probe:target hybrid with said  
*Ureaplasma urealyticum* target nucleic acid sequence under  
stringent hybridization assay conditions, wherein said *Urea-*  
*plasma urealyticum* target nucleic acid sequence is SEQ ID  
NO: 54: CAAUGGCUAA UACAAACUGC  
UGCAAAAUCG, and said hybridization assay probe tar-  
gets at least one nucleotide 5' to "A" at nucleotide position  
11 in SEQ ID NO: 54, wherein under said stringent hybrid-  
ization assay conditions said hybridization assay probe does  
not form a detectable probe:non-target hybrid with nucleic  
acid from *Mycoplasma hominis*.

\* \* \* \* \*